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TOME XCIX - Fasc. 2
Paru le 10 Avril 1961

PISA
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Journal of the International League against Epilepsy

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SYMPOSIUM ON BASIC MECHANISMS OF THE EPILEPTIC DISCHARGE

Held at the annual meeting of the American Electroencephalographic Society, on Cape Cod, Mass. (U. S. A.), June 11, 1960

Chairman: Arthur A. Ward, Jr., M. D.

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   Choh-Lu Li, Bethesda, Maryland.
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OFF-RESPONSES FROM THE AUDITORY CORTEX OF ANESTHETIZED CATS: EFFECTS OF STIMULUS PARAMETERS[1]

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Research Laboratory of Electronics,
Massachusetts Institute of Technology, Cambridge, Massachusetts, U.S.A.

INTRODUCTION

Electrical responses may be recorded from the auditory cortex of anesthetized cats very shortly after the termination of an acoustic stimulus (off-responses). Although many research workers have observed such off-responses and occasional mention of these observations can be found in several places, no report on a systematic study of these responses has been published, and little is known of their underlying mechanisms (2, 3). This paper is exclusively concerned with a study of *these* off-responses; it does not attempt to deal with the long lasting cortical after-effects of acoustic stimulation which have been investigated by Bremer (2) and others.

During a study of on-responses produced by various acoustic stimuli, we noted off-responses occasionally. We became curious about the extent to which the appearance of these off-responses is a function of stimulus conditions.

Thus far, the only clearly relevant statement that relates stimulus parameters to the evoking of off-responses deals with responses from the cochlea. Stevens and Davis discuss the behavior of the cochlear microphonic in relation to the terminal transients produced

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[1] This work was supported in part by the U. S. Army (Signal Corps), the U. S. Air Force (Office of Scientific Research, Air Research and Development Command), and the U. S. Navy (Office of Naval Research) and research grant B-1344, National Institute of Neurological Diseases and Blindness of the National Institutes of Health, Public Health, Service.

[2] Present adress: Lincoln Laboratory, Massachusetts Institute of Technology.

[3] Also at the Eaton Peabody Laboratory of Auditory Physiology, Massachusetts Eye and Ear Infirmary, Boston, Massachusetts.

when sinusoidal stimuli are abruptly turned off (12). They do not specifically mention what happens at the level of the auditory nerve, although they suggest the presence of "neurals" at the onset and at the termination of a 2.5 kc/s tone burst (12, Fig. 131). They do state, however, that these microphonic off-responses should occur when listeners report hearing a click at the end of a tonal stimulus. Stevens and Davis thus proposed a simple explanation for the production of these off-responses: that the off-response is a response to the click heard when a stimulus is abruptly terminated. Here, abruptly means, presumably, that the stimulus goes from peak amplitude to essentially zero amplitude in less than 5 msec.[1]

We felt that cortical off-responses, too, might be the reflection of terminal transients, a conjecture that we tried to verify by using various stimulus configurations to change the spectral content of the off-transients.

METHODS

The experimental subjects were 15 adult cats of 2,5 Kg average weight. Most of them were anesthetized with Dial (0.75 cc/kg body weight), although some were given nembutal. Cannulation was followed by opening the skull and the dura over the auditory cortex that was contralateral to the stimulated ear. An ear tube was tied into the external auditory meatus and connected to a PDR-10 earphone. Occasionally, the round window of the stimulated ear was also exposed so that a monitoring electrode could record the neural and microphonic potentials at that location.

The stimulus-generating equipment used in these experiments is shown in the block diagram of Fig. 1. Two electronic switches were used

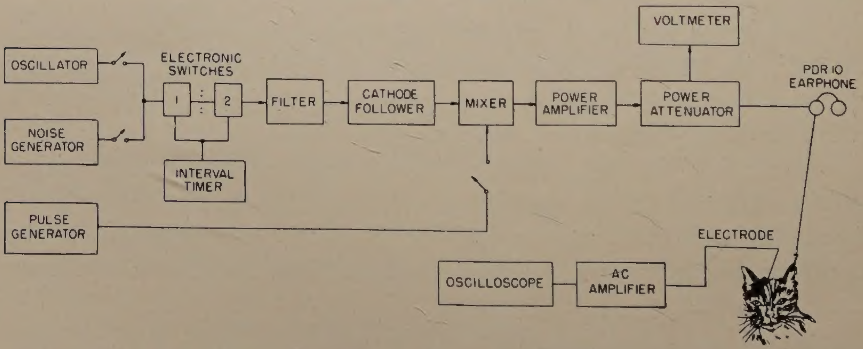

Fig. 1. – *Block diagram of stimulus-generating and recording equipment.*

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[1] The reader is referred to the discussion by Stevens and Davis of the acoustic characteristics of fast transitions in tonal stimuli (12) and to the more detailed discussion by Licklider of the frequency-intensity-time representation of such stimuli (9).

to shape the envelopes of the stimulus patterns (see Fig. 2). Thus we could vary onset times and decay times independently. The interval timer opens and closes the electronic switches by two pulses. One switch was set for fast onset and decay (10 μsec), the other for slower onset and decay (rise-fall) (5-25 msec). Since the same gating pulses open and close both switches, the effect of the combination of the two switches in series is to shape the envelope of the stimulus onset by means of the "slower" switch and to shape the decay of the stimulus by means of the "faster" switch. This combination thus makes it possible to produce an abrupt terminal transient at the end of a burst that has been turned on slowly.

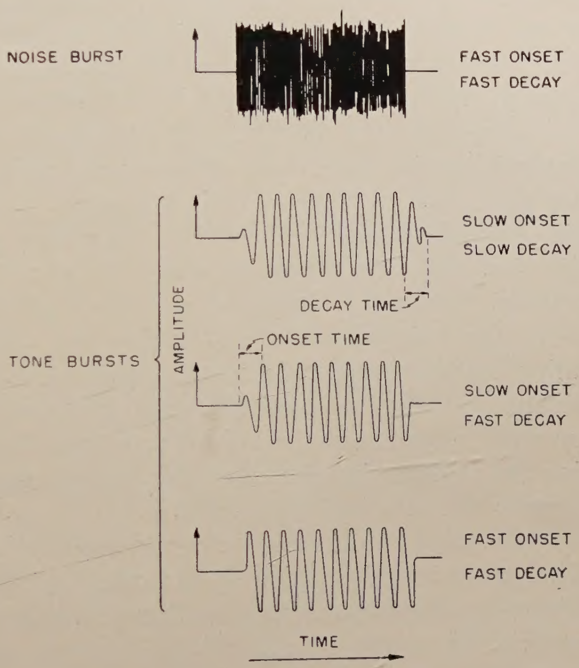


Fig. 2. – *Stimulus configurations.*

Because of the necessity of changing the spectra of the stimuli in these experiments, the stimuli were characterized by differences in onset time, decay time and carrier-frequency composition. Tonal stimuli were used when it was desirable to introduce marked transients at the beginning or end of the stimulus. Noise bursts were used when it was desirable to hold spectral content constant throughout the period of stimulation. Transients producing marked changes in spectra occur with tonal stimuli when onset or decay is fast. They are minimized when onset or decay is slow. With noise bursts, neither onset nor decay time is effective in producing spectral changes. It should be noted that these diagrams represent the electrical waveform at the earphone. Other transient characteristics in the stimulus will be introduced both by the earphone and the middle ear because of their particular resonant characteristics (1).

The electrical responses were recorded by concentric stainless-steel electrodes inserted into the primary auditory cortex (AI) with the sleeve resting on the cortical surface and the tip intruding approximately 1 mm into the cortex. The recorded potentials were fed to capacity-coupled amplifiers and displayed on an oscilloscope. The oscilloscopic traces were photographed with a Polaroid Land camera.

The experimental animal was isolated in a dark anechoic chamber, under conditions of controlled heat and humidity.

RESULTS

The experiments that were performed were designed to ascertain what stimulus conditions give rise to cortical responses at the termination of acoustic stimulation. Because we hypothesized that these off-responses are associated with changes in the instantaneous (or " short-term ") spectrum of the sound, we used stimulus configurations that are characterized by substantial changes in spectrum.

These spectral changes were produced in several ways. By controlling the onset and decay characteristics of bursts of tone we were able to produce various spectral changes at both ends of the stimuli. The " short-term " spectra ranged from wideband (for fast onset or decay) to narrow-band around the " carrier frequency " when the onset or decay was relatively slow. The spectra were also manipulated by changing the carrier frequency or by using bursts of noise as stimuli. Since an increase in the intensity of the stimulus (for constant onset and decay times) broadens the spectral range above the animal's absolute threshold without affecting the relative intensities of the various spectral components, intensity was also used as a variable.

Duration of tone or noise bursts was another stimulus parameter. Changes in burst duration do not affect spectra significantly within the range of durations used in these experiments, but such changes permit the study of interactions between on-responses and off-responses.

1. *Effects of onset and decay times*[1]. — Fig. 3 shows responses seen at the beginning and end of a tone burst as we vary onset and decay characteristics. For fast onset and decay, responses occur at both onset and termination of stimulation with the amplitude of the off-response smaller than the amplitude of the on-response.

The middle set of traces shows that the on-response is smaller for a 5-msec onset time than for a fast onset and that the off-response is larger here in relation to the on-response than when both onset and decay are fast.

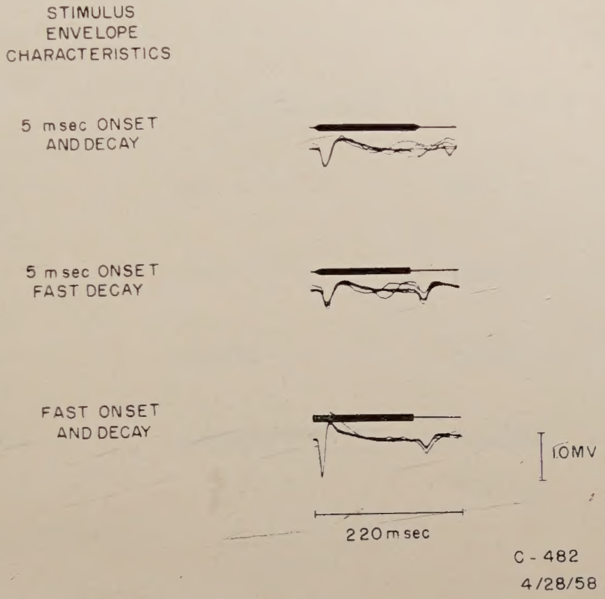
---

[1] The effect of onset time on both peripheral and cortical responses to noise bursts has been discussed elsewhere (4).

The upper set of traces shows that for onset and decay times of 5 msec, an on-response is present but there is no clear-cut off-response, although the variability of activity that can be observed at the end of the stimulus seems to exceed that seen in either of the other two traces.

Fig. 3. – *On- and off-responses to tone bursts. Effects of onset and decay characteristics.*

Upper traces show outlines of the envelope of the electric signal delivered to the earphone. Lower traces are 5 superposed recordings of the electrical activity of the auditory cortex (Middle AI). Tone bursts: frequency, 11.4 kc/s; intensity, −70 db; duration, 150 msec. " Fast " means an onset or decay time of 10 µsec. All intensities in these experiments are referred to 1 volt rms across the earphone.



2. *Effects of carrier frequency.* — Fig. 3 displayed responses to a tone burst of 11.4 kc/s. This frequency produced a response at the lowest intensity level for the particular cortical location of the recording electrode (middle AI). That the carrier frequency is important is illustrated in Fig. 4. The responses presented come from the same cortical location as those shown in Fig. 3. However, in Fig. 4 we see that a tone burst of 11. 4 kc/s clearly produces an on-response; a burst of 5.2 kc/s gives rise to a very small on-response.

Bursts of 5.2 kc/s and 11.4 kc/s when turned off abruptly both give rise to off-responses. The response observed at the *end* of the 5.2 kc/s tone burst (which produced the much *smaller* on-response) is clearly larger than that seen at the end of the 11.4 kc/s tone burst. This finding leads us to conclude that the carrier frequency is important in determining features of off-responses, in part, perhaps, through the mechanism of the on-responses of different amplitude.

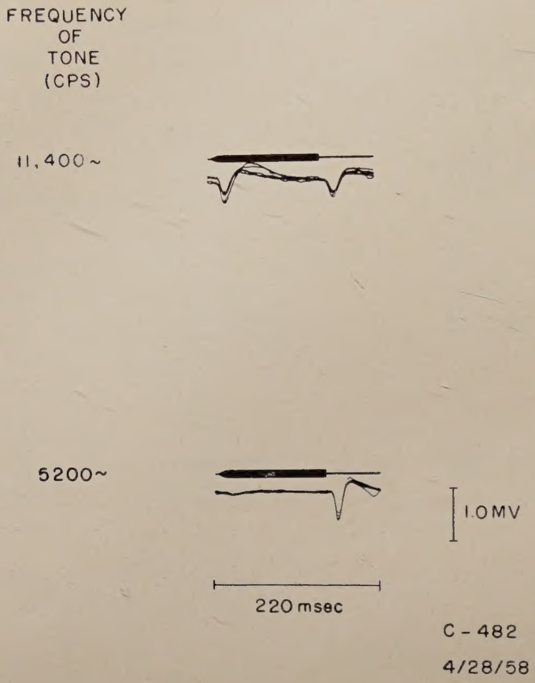


Fig. 4. – *Effects of carrier frequencies in the production of on- and off-responses for a given cortical location.*

Upper and lower traces correspond to those shown in Fig. 3, and are recorded at the same cortical location. Onset time, 5 msec; decay time, fast; intensity, –70 db.

3. *Composite effects of burst parameters: duration, onset time and carrier frequency.* — Onset and decay times and carrier frequency have been shown to affect the occurrence and size of the off-response. We now include these parameters in examining the effects of stimulus duration.

Fig. 5 displays on- and off-responses to bursts of tone which vary in duration, onset time, and carrier frequency. The rows show the effects of duration; and the columns, the effects of carrier frequency and onset characteristics. The two frequencies were chosen because at these stimulus intensities and onset times, the 1. 25 kc/s tone bursts produced bigger on-responses than the 4.0 kc/s tone bursts.

Duration of the tone burst appears to affect off-responses particularly when the stimuli are characterized by fast onsets (left column) that give rise to sizeable on-responses, even for a 4.0 kc/s tone burst.

The traces in the top row of Fig. 5 show that for equal durations (60 msec) of the tone bursts off-responses are clearly visible when the onset is slow, that is, when the on-response is relatively small or hardly detectable, but not when onset is fast. As the duration of the bursts lengthens to 120 msec, clear off-responses are observed for all stimulus conditions illustrated.

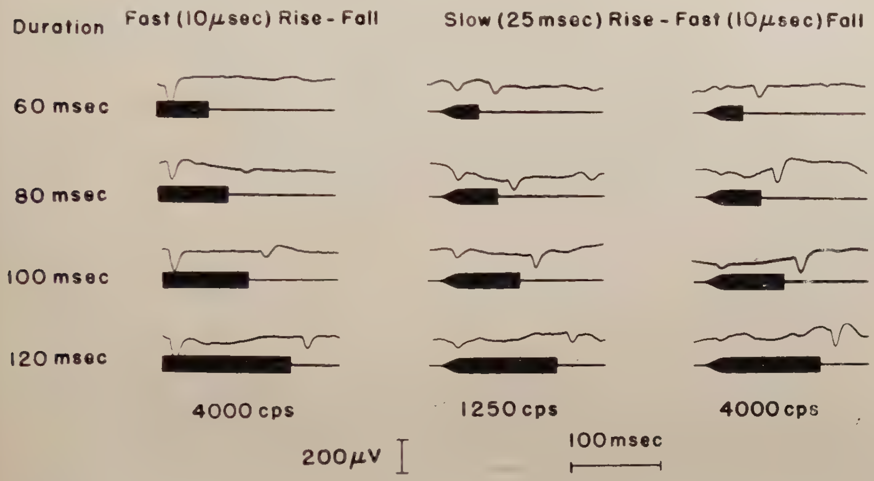


Fig. 5. – *Composite effects of various stimulus parameters.* Intensity, –70 db.

4. *Off-responses to wideband noise bursts.* — Our initial hypothesis and the data presented thus far would lead us to predict that noise bursts should not produce off-responses. Wideband noise contains all frequency components that the acoustic system will deliver to the inner ear. Therefore, no new frequency components could appear instantaneously as the stimulus is turned off. Fig. 6 illustrates the responses recorded for noise bursts of different intensities. It can be seen that off-responses simply do not occur at any intensity with this stimulus, even though on-responses are clearly present.

The data of Fig. 6 can be extended. We find that longer noise bursts fail to produce off-responses. This result again focuses our attention upon the contents of the envelope of the stimulus configuration. As we have seen, off-responses interact with on-responses. Undoubtedly, they are also affected by the activity generated in the auditory system by the stimulus that is present throughout the duration of the burst, and, in particular, by what is present in the stimulus envelope immediately before the stimulus is turned off.

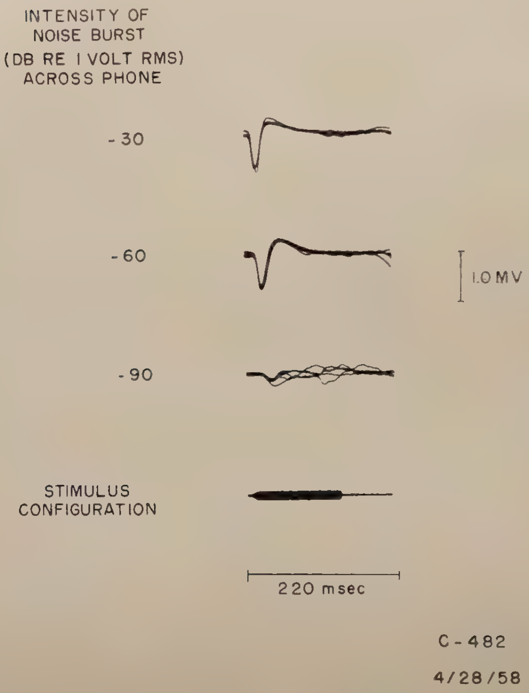


Fig. 6. – *On- and off-responses to noise bursts of 150 msec duration.*

Recordings from the same location as in Figs. 3 and 4. Five responses are superposed to produce each trace. Onset and decay time, fast.

5. *Effects of stimulus intensity.* — Thus far, we have not considered explicitly the parameter of stimulus intensity. In Fig. 7 two intensity series are presented from a cortical location exhibiting on-responses to low-intensity tone bursts of 9.0 kc/s, but not of 2.0 kc/s. The on-response to the slow onset of the 9.0 kc/s stimulus appears clearly at -100 db. The off-response to the fast decay appears only at -80 db. This characteristic difference has been observed in all of the seven animals that were studied with respect to intensity [1].

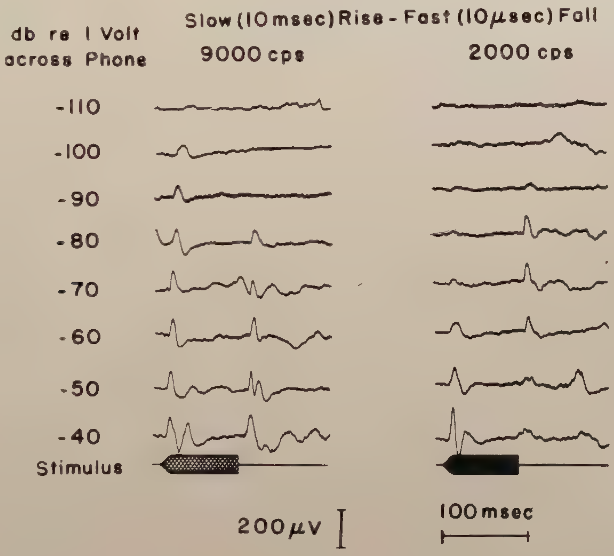
---

[1] This relation seems to hold only for carrier frequencies that give rise to on-responses at the lowest stimulus intensity. This effect may thus be related to the tonotopic organization of the cortex (6, 7, 13).

With the 2.0 kc/s tone burst the pattern is quite different. For this frequency, the off-response appears at nearly the same intensity as the off-response to the 9.0 kc/s stimulus. However, the on-response does not appear until the intensity level of the tone reaches approximately –75 db.

Above –60 db, a rather remarkable phenomenon occurs. The on-response to the 2.0 kc/s tone burst grows larger and the off-response grows smaller. For 9.0 kc/s tone bursts, however, the off-responses do not diminish in size; on the contrary, they increase with stimulus intensity just as the on-responses do.

Fig. 7. – On- and off-responses to tone bursts of different carrier frequencies for an intensity range of 70 db.



Intensity functions for both on- and off-responses for different carrier frequencies are shown in Fig. 8. All responses were recorded from the same cortical location, which proved maximally sensitive to tone bursts of approximately 11.4 kc/s. At this frequency, a small off-response is still present for the greatest stimulus intensity presented. At all other frequencies, on- and off-responses appear at higher intensities than for 11.4 kc/s. For the lower frequencies (5.0 kc/s, 1.14 kc/s, and 0.5 kc/s) the off-response is not present at high intensities, but for 17.0 kc/s there remains a sizeable off-response, even at 0 db. These results indicate that off-responses depend not only on carrier frequency and cortical location but also on intensity. Clearly, such phenomena must be examined within the framework of a multidimensional model, since the effects of intensity, duration, onset-decay times, carrier frequency and cortical location are all interrelated. In the following discussion we shall try to summarize some of these relations in terms of some simple models.

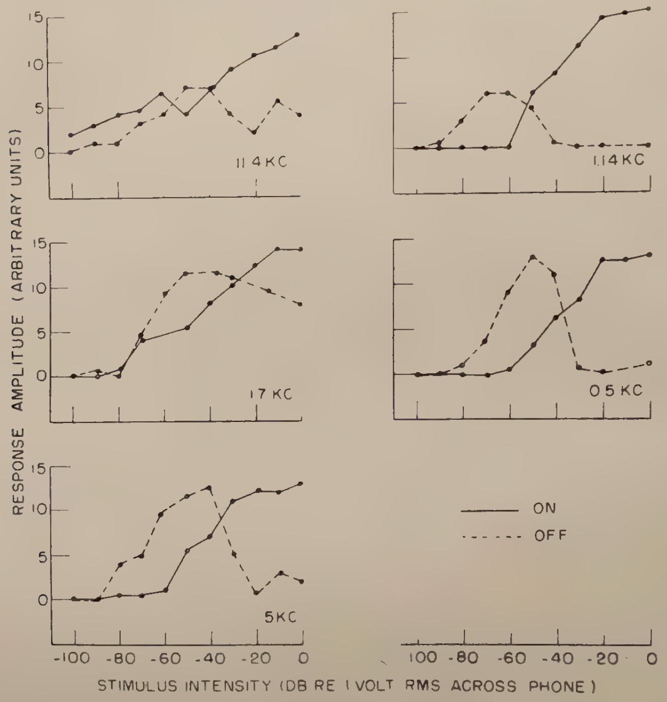


Fig. 8. – *Response amplitude for on- and off-responses versus stimulus intensity for different carrier frequencies from a single cortical location.*

These functions were plotted from traces like those shown in Fig. 7. The measured variable was the peak amplitude of the initial deflection of the responses.

Discussion

The main experimental evidence presented here can be summarized as follows. The off-responses in anesthetized cats are, in fact on-responses; these on-responses are evoked by the new spectral components that appear in the short-term spectrum of a rapidly decaying stimulus.

We may picture a hypothetical auditory cortex in which each point is characterized by " response areas " or " tuning curves "; this means that each cortical patch is maximally sensitive to a particular frequency (6, 13). We may assume that a given frequency component of a stimulus activates its corresponding cortical patch, and that this activation modifies, that is, temporarily decreases, the responsiveness of the patch.

Fig. 9 shows diagrammatically four conditions of this hypothetical auditory cortex at onset and decay of both a tone burst and a noise burst. The top diagrams represent the state of activation at onset, and the bottom diagrams represent the cortical areas that are activated by the spectral components in the off-transient. While we have not systematically studied the electrical events that follow the on-response and precede the off-response, the observations reported support the view that the cortical areas influenced throughout the duration of the acoustic stimulus substantially reflect the spatial pattern of activation that characterizes the on-response.

Let us consider these patterns of activation. The degree of activation is shown by the blackness of the cortical surface; white surfaces indicate areas that have not been activated. The pattern of activation on the cortex is quite restricted for a tone burst with slow onset, but at the onset of a noise burst when all frequencies are present in the stimulus, the entire auditory cortex is activated as shown in the upper right diagram of Fig. 9.

At the termination of the tone burst the cortex responds moderately to the broadband transient produced by the abrupt termination. Here, activation covers the entire auditory cortex with the exception of the area covered by the black dot in the upper left diagram, since this area is in a less responsive phase. With the noise burst, however, no synchronized activity is seen anywhere in response to the termination; no new spectral components have been introduced.

How does this hypothetical model compare with our experimental results? When both onset and decay of the tone bursts were fast, both on- and off-responses were observed. With weak tone bursts whose onset is slow only a small extent of cortex will be involved in the on-response. Thus the off-response is of maximum amplitude for a rapidly decaying tone burst that gives rise to many new spectral components.

Fig. 3 shows that a slowly decaying tone burst fails to produce

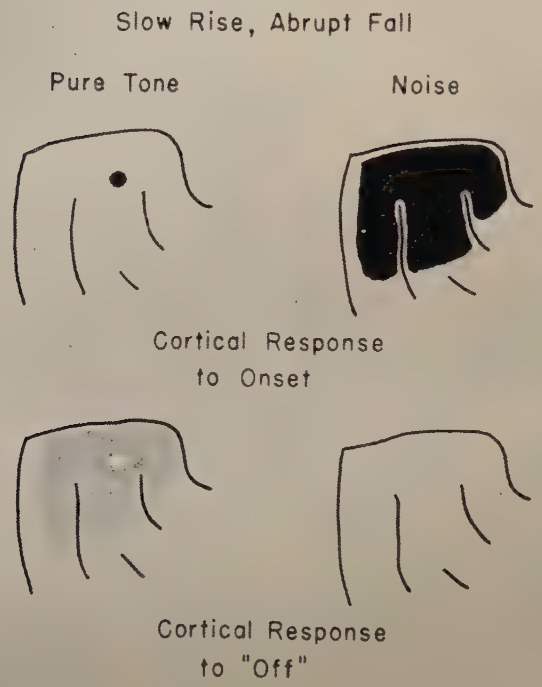


Fig. 9. – *Hypothetical diagram of activation of the auditory cortex with tone and noise bursts used as stimuli.*

The stimuli are characterized by 5 msec onset and fast decay times. (See Fig. 2). Degree of blackness of cortical surface indicates degree of activation, as shown by synchronized evoked potentials. White denotes no activation. The stimuli are presented at moderate intensity levels.

an off-response; the absence of new spectral components gives rise to no additional cortical activation.

The assumption that activation at any specific cortical location is restricted to a certain frequency range when low intensity tone bursts are turned on slowly is clearly demonstrated in Fig. 4. For an appropriate carrier frequency, an evoked response appears, even for slow onsets. However, at low stimulus intensities off-responses are never seen with slowly decaying stimuli in the anesthetized cat, regardless of the frequency of the tone bursts. To this extent, on-responses differ from off-responses. This difference is attributable to the fact that at the onset of the tone, a spectral component appears, whereas at the termination, that component simply disappears.

Tone bursts with fast onset and decay can be compared with two click experiments in which the time-delay between the clicks is the variable (11). The fast onset-decay experiments show that the behavior of off-responses resembles the behavior of the response to the second of two clicks. There are, however, differences between the present experiments and two-click experiments. These differences appear when the carrier frequency is the frequency for which the cortical location is " tuned ", that is, has the lowest threshold. In this instance the off-response is consistently of smaller amplitude than off-responses for other carrier frequencies. Within the framework of our model, this finding could be accounted for by reduced responsiveness of the cortical patch in the immediate vicinity of the recording electrode. This reinforces the view that the cortical patch would have been in a state of continuous, but not synchronous, activity throughout the duration of the tone burst.

When noise bursts are used as stimuli no off-responses are ever observed, regardless of the values of the various stimulus parameters. This finding is, of course, in complete agreement with the prediction of our undoubtedly oversimplified model.

Some difficulties arise in fitting our model to the data of Figs. 7 and 8. These data depict intensity functions for on- and off-responses to tone bursts with slow onset and fast decay. The on-response for such stimuli defines the " tuning curves " (6, 13). In Fig. 7, the fact that the cortical location is obviously more sensitive to 9.0 kc/s than to 2.0 kc/s is shown by the appearance of an on-response at a lower intensity for the higher frequency. In Fig. 8, the cortical location is more sensitive to 11.4 kc/s than to any other frequency. For all frequencies, on-responses and off-responses grow as stimulus intensities increase, to some degree. However, for the frequency range of maximal sensitivity the off-response levels off, while the on-response continues to grow with increasing stimulus intensity. For frequencies that are remote from this region of maximal sensi-

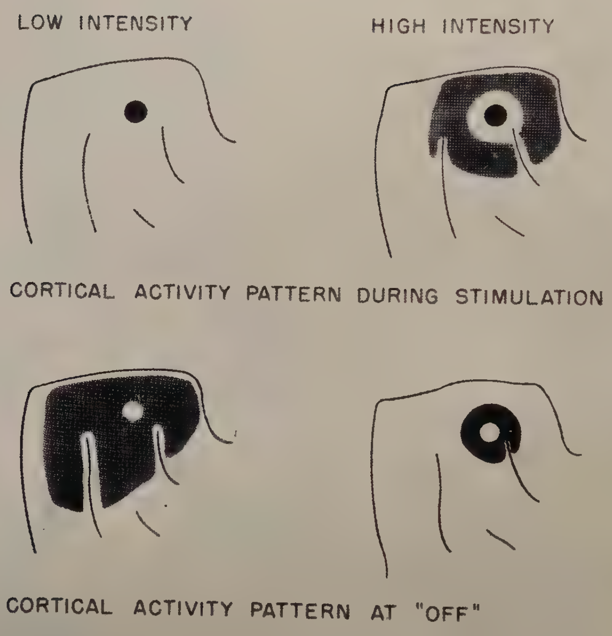

LOW INTENSITY
HIGH INTENSITY
CORTICAL ACTIVITY PATTERN DURING STIMULATION
CORTICAL ACTIVITY PATTERN AT "OFF"

Fig. 10. – *Cortical response areas under conditions of low- and high-intensity stimulation.*

The stimulus consists of a tone switched on with 5 msec onset and fast decay. The black areas represent regions of intense activation; the gray areas, of somewhat less activation. The white areas are devoid of activation, either because of inhibition or tonotopic organization. In the upper right diagram, the white annulus around the black dot represents an area devoid of activity because of inhibition, probably at some lower area in the auditory tract; in the upper left diagram the white area represents a region devoid of activity because of the restricted local excitatory capability of the narrow-band carrier frequency used as stimulus.

tivity, the off-response decreases to zero for strong stimuli, while the on-response grows significantly for medium and high intensities. Apparently, the intensity functions of off-responses depend strongly on the carrier frequency and cortical location. According to our model, the frequency at which the on-response would most effectively occlude the off-response should be the frequency to which a cortical location is " tuned ". The data, unfortunately, show that exactly the opposite is the case.

While there are many possible explanations of such a phenomenon, the one that appeals to us most is this. Assume a hypothetical auditory cortex in which each active patch is surrounded by an inhibitory halo. This model has tonotopic organization, so that a weak pure tone results in excitation at the location that is in " tune ". As the stimulus intensity is increased, larger areas of cortex are activated, as shown by the findings that yield response areas. However, there is a local ring of inhibition near the location that is in tune. At the termination of high-intensity tone burst, the " untuned " locations are in a state of diminished responsiveness, and hence do not respond. The inhibitory ring is released from inhibition at the termination of the bursts and responds to the transients (see Fig. 10).

A similar concept of an inhibitory ring has been proposed by Mountcastle for the somatic cortex on the basis of single-unit data (10). Our model is, of course, clearly inspired by the demonstrations of inhibitory neural interactions furnished by Hartline, Wagner, and MacNichol (5), and by Kuffler (8).

Even our slightly more complicated model is obviously too simple for predicting the effects of interactions of responding elements when complex stimulus patterns are introduced. Our model does serve as a convenient summary of the more important results presented and provides hypotheses for further experimentation.

SUMMARY

The effects of various stimulus parameters in evoking auditory off-responses has been investigated. It has been found that off-responses will occur when the terminal transient of the stimulus covers a broad band of frequencies. A simple model has been proposed to explain the phenomena as they occur with low-intensity stimuli.

Another model has been proposed to account for off-responses that are seen when high-intensity stimuli are used. This latter model incorporates the idea of an inhibitory "halo" surrounding an area of intense local activation of the cortex.

ACKNOWLEDGMENTS

The authors wish to acknowledge the encouragement and many helpful suggestions of Professor W. A. Rosenblith, of Massachusetts Institute of Technology. It is also a pleasure to express appreciation for the technical assistance of the Staff of the Research Laboratory of Electronics, Massachusetts Institute of Technology, in particular, to Mr. R. M. Brown.

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Arch. ital. Biol., *99*: *105-120*, *1961*.

OFF-RESPONSES FROM THE AUDITORY CORTEX OF UNANESTHETIZED CATS[1]

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Massachusetts Institute of Technology, Cambridge, Massachusetts, U.S.A.

INTRODUCTION

In a previous paper (17), we have described electrical responses from the cortex of anesthetized (Dial) cats at the termination of a tone burst. These responses are generated by additional spectral components in the acoustic stimulus, which occur whenever a tone is turned off. As we have shown, we were not able to obtain responses to the termination of bursts of wideband noise, because the termination of such a burst does not produce any new frequency components. These observations are consistent with reports from listeners: if tone bursts or noise bursts are turned off abruptly, one hears a distinct click at the end of the tone bursts, but not at the end of the noise bursts. The present paper reports a second type of off-response. This response is found in unanesthetized preparations only, and appears to be generated by a different mechanism.

METHODS

All of our experiments were conducted in a soundproof anechoic chamber under controlled temperature and humidity conditions. Fifteen cats were used in this study. They were immobilized by a high spinal section performed under ether; the section was at either C_1 or C_2. A plastic tube connected to a PDR-10 earphone was inserted into the external auditory meatus. The contralateral auditory cortex was exposed and gross concentric electrodes (17) were placed in selected areas of the auditory cortex. A block diagram of the stimulus-generating equipment and recording apparatus is shown in Fig. 1.

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[1] This work was supported in party by the U. S. Army (Signal Corps), the U. S. Air Force (Office of Scientific Research, Air Research and Development Command), and the U. S. Navy (Office of Naval Research) and Research grant B-1344, National Institute of Neurological Diseases and Blindness of the National Institutes of Health, Public Health Service.

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Because the cortical activity of unanesthetized cats exhibits considerable " spontaneous " activity that interferes with the study of evoked responses, we found it desirable to record the responses on magnetic tape and, subsequently, to obtain average responses by using electronic computers. This method has been described previously (2, 6). Six tape-recorder channels were usually available. These were used to record: (*a*) activity at the surface of AI referred to the headholder; (*b*) activity at the surface of AI referred to activity recorded 1.5 - 2.5 mm below the surface of the cortex (" depth electrode "); (*c*) activity at the surface of AII referred to the headholder; (*d*) activity at the surface of AII referred to the " depth electrode "; (*e*) gating pulses synchronized with the stimulus onset; and (*f*) gating pulses synchronized with the beginning of the decay of the stimulus. These pulses provide reference time markers in the averaging process for on- and off-responses.

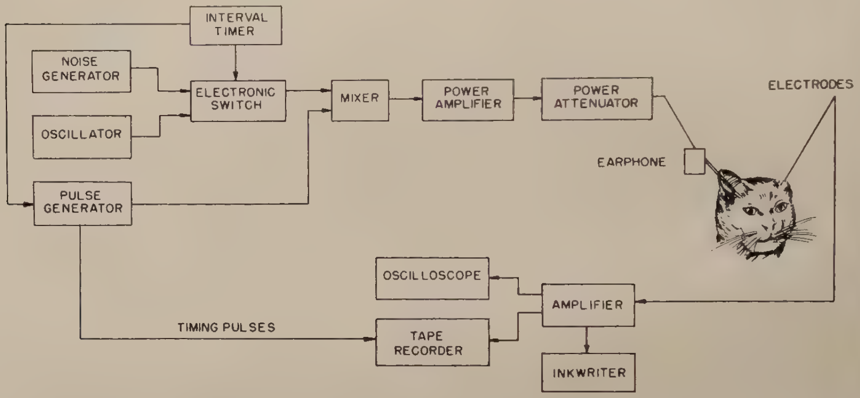

Fig. 1. – *Block diagram of stimulus-generating and recording equipment.*

Responses were usually recorded under various stimulus conditions while the preparation was not anesthetized. The animal was then given anesthesia, either by injecting nembutal into the femoral vein, or, more frequently, by injecting Dial into the peritoneal cavity. After anesthesia had been administered, the electrical activity of the cat's auditory cortex was again recorded for the same stimulus conditions. All responses that belong to a series (see Results) were obtained in successive runs; this procedure minimized any long-term physiological changes.

RESULTS

The present investigation began during a study of evoked cortical responses to bursts of noise in unanesthetized cats. We noted that prominent electrical events occurred after the noise bursts had been turned off. Earlier, we had been unable to obtain evoked responses to the termination of noise bursts in anesthetized animals (17). Therefore we concluded that these responses from unanesthetized cats differed in kind from the off-responses previously observed. Subsequent attempts to find these off-responses in unanesthetized cats were not consistently successful until we noticed that in our " successful " experiments the recording electrodes were in Middle AII. Since that discovery, we have recorded off-responses to bursts of noise regularly from Middle AII of unanesthetized animals. The repeatability of this phenomenon made systematic study possible.

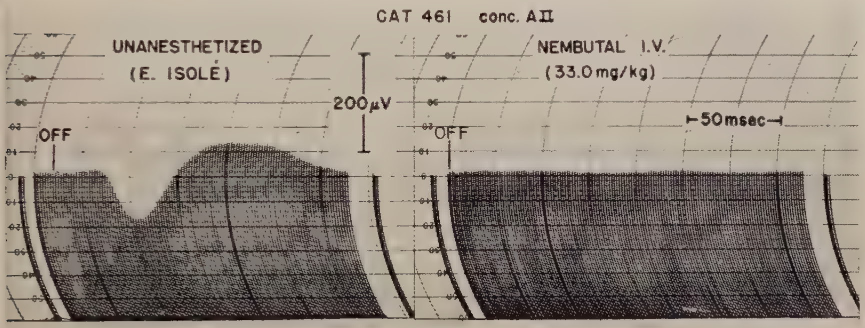

Fig. 2. – *Effects of intravenous nembutal on the off-response.*

Each curve is the computed average of 300 responses to bursts of noise. Pen deflections are separated by 1.25 msec intervals. Upward deflection represents surface negativity. Stimulus: bursts of noise with 10 msec onset and decay time (rise-fall time), 375 msec burst duration, 1 burst per sec, Intensity, 60 db re VDL (visual detection level) for on-responses.

1. *Effect of anesthesia.* — We had to establish, first, that the animal's state of anesthesia was the critical factor in the production of off-responses to bursts of noise. We prepared a series of animals with spinal sections and recorded responses both before and after the administration of anesthesia. In these experiments, nembutal was injected into the femoral vein while the responses were being continuously observed and recorded. Within a minute after the injection of nembutal, the off-responses were completely abolished, but the on-responses showed the typical waveform of evoked responses in nembutal-anesthetized cats. Fig. 2 shows these results in the

form of computed average responses for the same cat. The average response on the left was computed from an *encéphale isolé* preparation; the average response on the right came from the same cat after intravenous injection of nembutal. The effect of anesthesia proved reversible. When an anesthetized animal was permitted to recover from anesthesia, the off-responses to bursts of noise reappeared. Thus, it is clear that it is the existence of off-responses to bursts of noise which distinguishes the two states of the preparation. We decided to vary the same stimulus parameters that had been varied in the earlier study: decay time, duration, intensity, and repetition rate. Here, these parameters are applied to noise bursts.

2. *Effect of decay time.* — Varying the decay time leaves the off-responses essentially unaffected. Fig. 3 shows average responses for a fast decay and for a 10-msec decay. The latencies of character-

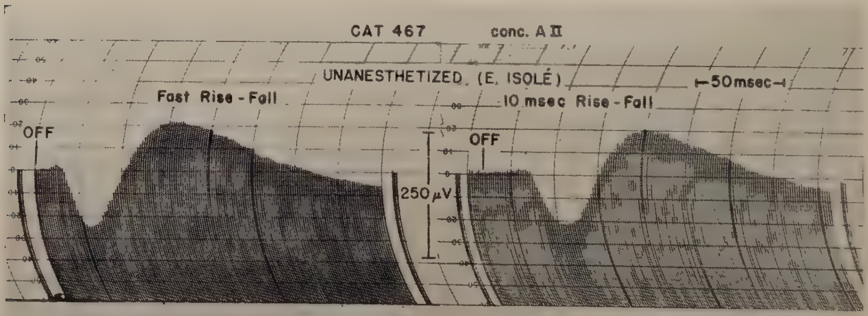

Fig. 3. – *Effects of changing onset-decay time on the off-response.*

Each curve is the computed average of 300 responses. Stimulus: bursts of noise of 500 msec duration, 1 burst per sec. Intensity, 40 db re VDL for on-responses. Fast onset-decay is approximately 10 µsec for the electric imput to the earphones. Off-markers indicate the times at which the decay of the electric signal starts. Latencies of this type of off-response: for fast decay, approximately 15 msec to onset, 30 msec to the surface positive peak, and approximately 73 msec to the surface-negative peak; for the 10 msec decay, the corresponding figures are approximately 30, 50, and 90 msec.

Fig. 4. – *Effects of changing duration of noise bursts on off-responses.*

Each curve is the computed average of 300 responses. Stimulus: bursts of noise with fast onset-decay, 1 burst per sec. Intensity, 40 db re VDL for on-responses.

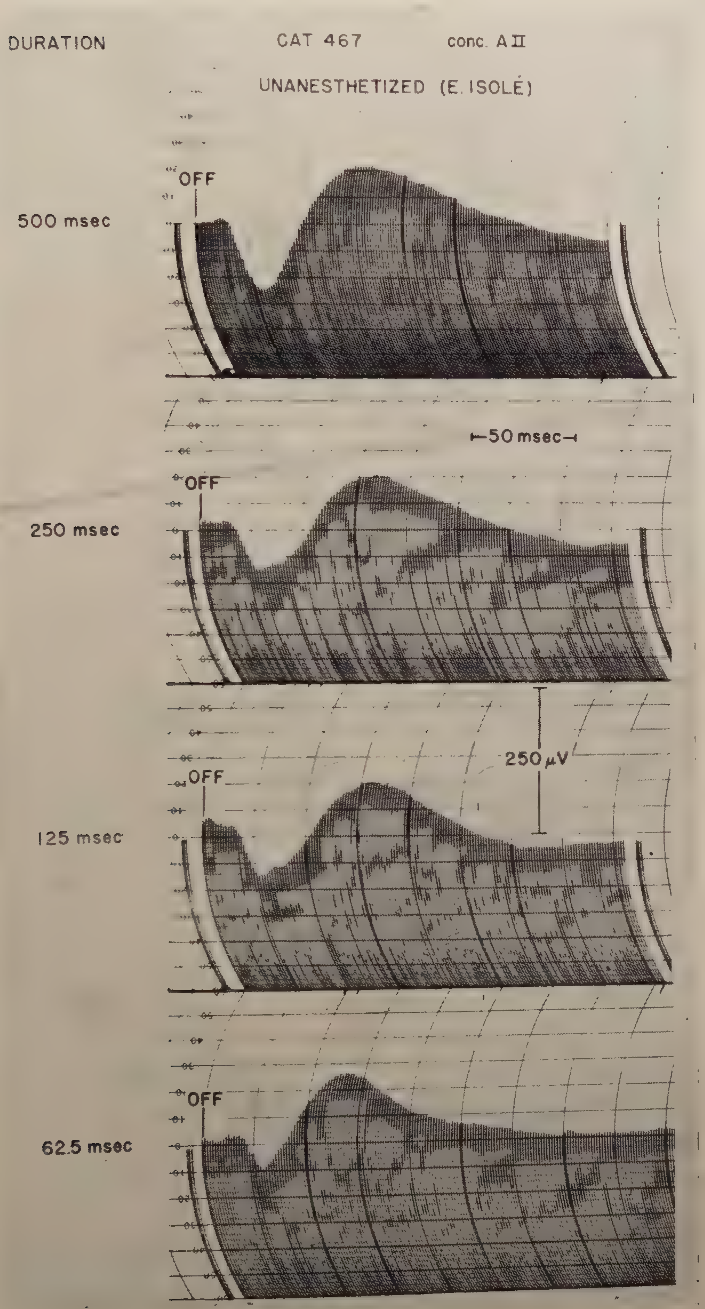
DURATION
CAT 467
conc. A II
UNANESTHETIZED (E. ISOLÉ)
OFF
500 msec
⊢50 msec⊣
OFF
250 msec
250 µV
OFF
125 msec
OFF
62.5 msec

Fig. 4.

istic points in the evoked response are substantially longer for the 10-msec decay time. This increase in the latency cannot be attributed to any single factor, and we shall therefore have to postpone interpreting it now. The waveform of the average response is also slightly broadened for the 10-msec decay time. This broadening may be attributed to a decrease in synchrony of the underlying neural activity (5).

Clearly, these off-responses in the unanesthetized cat are less affected by changes in decay time than the off-responses found in the anesthetized cat are. It will be recalled that, for comparable stimulus intensities, off-responses to tone bursts are not seen in anesthetized animals when the decay time exceeds 5 msec.

3. *Effect of duration of stimulus.* — As the duration of the burst of noise is shortened, the off-responses decrease in amplitude and change in waveform (Fig. 4). For durations as short as 50 msec (Figs. 5 and 6), off-responses are still present ; however, in anesthetized animals off-responses to tone bursts with fast onset and decay are no longer found for durations as short as this. This last finding parallels results from two-click experiments in anesthetized cats.

4. *Effect of intensity of noise bursts.* — The amplitude of the surface positive deflection of off-responses to noise bursts increases with the intensity of the bursts (Fig. 5). Changes in the waveform and latency of these responses with intensity will not be considered here. The visual detection level of these off-responses is very nearly the same as that for the on-responses. Once both types of responses are present, their amplitudes may behave quite differently. In anesthetized cats there is usually a difference of 20 db between VDL's for on- and off-responses to tone bursts (17).

5. *Effect of repetition rate of noise bursts.* — The off-responses to noise bursts are rather sensitive to the rate at which these bursts

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Fig. 5. – *Effects of changing intensity of noise bursts on off-responses.*

Each curve is the computed average of 300 responses. Stimulus: bursts of noise with fast onset-decay, 50 msec duration, 1 burst per sec. Intensities are expressed in terms of db re VDL. When the switching transients alone were delivered to the animal, no responses were detectable, even at 40 db re VDL. At higher intensities, it is no longer possible to discount the effects of switching transients.

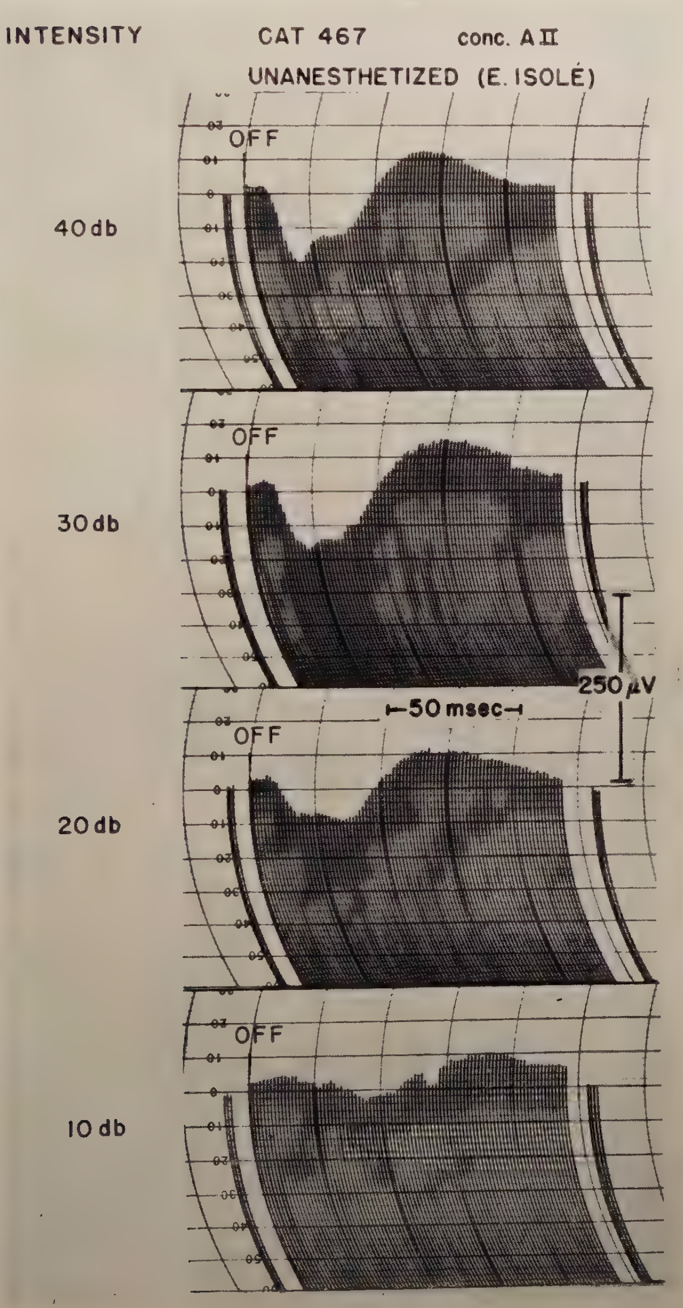
INTENSITY
CAT 467
conc. A II
UNANESTHETIZED (E. ISOLÉ)
OFF
40db
OFF
30db
250 µV
←50 msec→
OFF
20db
OFF
10db

Fig. 5.

are repeated. Whereas on-responses to bursts of noise (with fast onset) can be found for rates as high as 200/sec (6), off-responses in unanesthetized animals vanish for all practical purposes, for rates as low as 10/sec (Fig. 6).

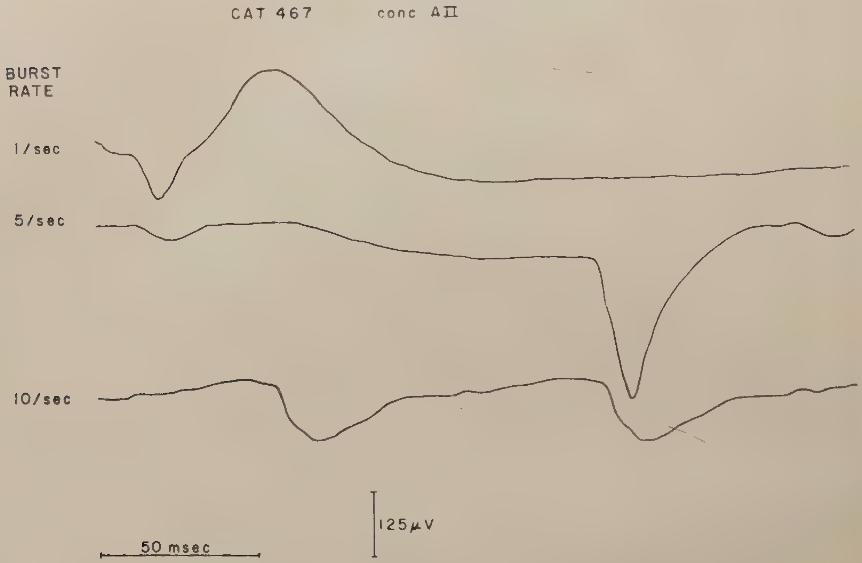

Fig. 6. – *Effects of repetition rate of noise bursts on off-responses.*

Each curve is a tracing of the computed average of 300 responses. The time scale has been chosen so that the on-responses following each off-response have also been averaged and displayed. Stimulus: bursts of noise with fast onset-decay, 50 msec duration. Intensity: 40 db re VDL.

An interesting result is obtained when bursts of chopped noise are the stimuli (Fig. 7). There is, at first, a large response to the

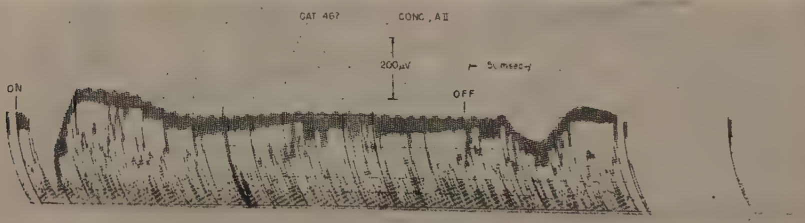

Fig. 7. – *Average response to bursts of chopped noise.*

Stimulus: bursts of chopped noise with 3 msec onset-decay time, 375 msec duration, 1 burst per sec. Intensity, 40 db re VDL for on-responses. The chopping rate is 100/sec with 50% duty cycle and fast onset-decay onset of the series of noise bursts, then there is a series of " small " responses at the chopping rate (100/sec), and, at the end of the entire burst, a large off-response [1]. These findings are consistent with previous work on steady-state responses to chopped noise (6).

6. *Off-responses from the cochlea.* — When a gross electrode is placed on the bone of the cochlea near the round window, potentials that represent the synchronized discharge of many auditory nerve fibers can be recorded. Transient stimuli such as clicks produce the classic N_1 and N_2 configurations. If a burst of tone or noise with rapid onset time is used, a similar configuration is found (5). It is generally thought that evoked potentials at the auditory cortex depend on a burst of neural activity at the periphery of the system. Such a burst may, or may not, be organized into an N_1 component (5). Fig. 8 shows that no N_1 and N_2 configurations can be dectected in the peripheral responses to the termination of bursts of noise.

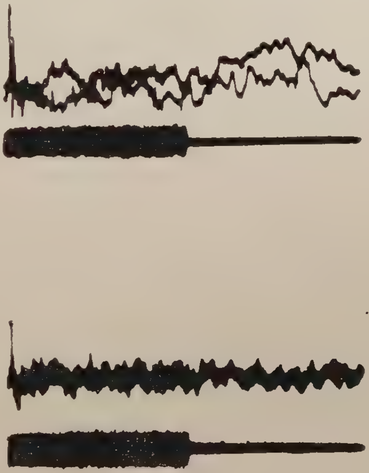

Fig. 8. – *Peripheral responses to bursts of noise.*

Spinal-sectioned preparation with electrode near round window. In each picture, upper traces show the peripheral responses; lower traces, the stimuli. Stimulus: bursts of noise with fast onset-decay, 100 msec duration, 1 burst per sec. Intensity, 30 db re VDL for N_1 response at the onset. The " spikes " at the onset of each burst of noise are the N_1 and N_2 components. No such spikes are seen at the termination of the noise bursts. The upper set of responses was recorded with a highpass filter setting at 8 cps. The bottom set of responses was filtered (highpass filter setting at 100 c/s), in order to remove the low-frequency potentials.

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[1] It is known that dc shifts occur in the auditory cortex as a result of continuous acoustic stimulation (13). Since we were using capacity-coupled amplifiers, it might be suggested that the off-response is the response of the amplifiers to the return of the cortical dc potential to its resting level. This is not the case. If the highpass filters are set as low as 0.5 c/s, the recorded responses are clearly too fast to be " amplifier return ". Changing the highpass cutoff frequency should change the form of the response if the response involved " amplifier return ", but we found no change in the responses when the filter settings were changed from 0,5 to 8.0 cps.

DISCUSSION

The experiments presented here, together with our previously reported results (17), demonstrate conclusively that off-responses to noise bursts in unanesthetized cats are not responses to the introduction of additional spectral components. Before we can suggest a possible mechanism for these off-responses, it may be appropriate to examine them in some detail. The duration of the off-response, which exceeds even that of the evoked on-response, certainly rules out the possibility that these off-responses result from actual summation of spike potentials from single units. It is far more likely that these responses are summed " slow potentials " generated within the cortex. It is axiomatic, however, that these slow potentials must be initiated by more or less synchronized bursts of spikelike activity which constitute the input to the cortex. The fact that no N_1-like off-responses can be recorded from the periphery at the end of noise bursts suggests that the auditory nerve does not deliver synchronous bursts when the noise is turned off. Throughout the duration of the burst, the neural elements may have been sufficiently desynchronized to render a synchronous off-response either unlikely or undetectable by macroelectrodes. A similar activation has been described for on-responses to noise bursts at the periphery (5). Both cortical on-responses and cortical off-responses are broader in waveform than the N_1 and N_2 potentials recorded from the auditory nerve, and thus can be assumed to be more resistant to such desynchronization. Single-unit recordings, both at the periphery and at the cortex, would help to clarify what actually happens.

Nevertheless, it may be proposed that cortical off-responses to noise bursts are correlated with a cessation of activity at the periphery. A simple conceptual model might involve the following mechanism:

i) A burst of noise turned on rapidly gives rise to a synchronized burst of neural activity in the auditory nerve. This neural burst eventually results in a cortical " on-response ".

ii) As long as the noise burst continues there is continuous asynchronous activity in the auditory nerve. This peripheral activity results in (*a*) excitation of certain units at higher levels of the nervous system, and (*b*) inhibition of " on-going " activity in other units.

iii) When the burst of noise is turned off, the activity in the auditory nerve ceases. The units that had been previously inhibited are suddenly released, and the synchronous return of " on-going " activity gives rise to a cortical off-response.

On this simple framework it is possible to hang the results that we have obtained. With the present evidence it is difficult to pinpoint particular anatomical locations at which units are inhibited, Presumably, either the inhibited neurons or the neurons whose activity they affect are more sensitive to barbiturate anesthesia than the units that give rise to on-responses. We can offer no interpretation for the observation that off-responses to noise bursts can be found more easity in Middle AII than in AI, except that this finding may be related to differences in recovery time between AI and AII as revealed by the two-click studies of Perl and Casby on anesthetized cats (14).

Except for Galambos' study of the medial geniculate body (4), there are few data on off-responses at intermediate levels of the auditory system. Activity of the entire primary pathway, as well as of the reticular formation in response to bursts of noise, needs to be studied.

What is the possible functional significance of the off-responses to noise bursts in unanesthetized animals? Such off-responses do not follow stimuli presented at rates as low as 10/sec. Perhaps such responses do not convey information about any particular characteristics of the stimulus itself but, instead, represent the general state of stimulation. Accordingly, when long bursts of chopped noise are used, the off-response comes at the end of the entire burst, thereby indicating the end of a state of stimulation.

Off-responses in the auditory system have been described by a number of workers (1, 3, 4, 10, 12). Several studies relate to the off-responses described in this paper. (Recall that we found such responses only in unanesthetized animals). Galambos has reported off-responses in single units at the level of the medial geniculate body to slowly decaying bursts of tone (4). His animals were " anesthetized with dial-urethane, the dose being adjusted to prevent motion of the preparations " (16). At the auditory cortex, single-unit responses to the termination of bursts of tone with slow fall times have been reported (10, 12). It is perhaps significant that these preparations were either unanesthetized or rather lightly anesthetized.

We may compare the auditory off-responses with off-responses observed in other sensory systems. For visual stimuli, there is no problem of spectral spread at the start and termination of stimulation, since the onset and decay times are usually very much longer than the period of an electromagnetic vibration.

It is well known that the electroretinogram shows a distinct off-response at the cessation of continuous photic stimulation. Visual off-responses have been studied in single units at the periphery of both invertebrates and vertebrates. The main features of these off-responses have been summarized by Granit (7), who also reviews the findings that show the importance of inhibition in the production of off-responses.

Ratliff and Mueller (15) have "synthesized" off-responses in individual Limulus optic-nerve fibers by manipulating the stimuli so as to combine inhibition by neighboring elements with after-discharge of the "off" fibers. Our conception is similar to theirs, in the sense that inhibition plays a key role. They suggest that the off-responses that they observed are after-discharges from units whose activity during stimulation had been suppressed by interaction with other units. We cannot be as specific as that because we have no data to indicate whether cortical off-responses to noise bursts represent synchronous afterdischarges or synchronized returns to "on-going" activity.

One difficulty in studying off-responses in the periphery of the visual system is that it is difficult to isolate responses from first-order neurons in the absence of interaction. For vertebrate eyes, it is certainly safe to say that off-responses occur in structures as peripheral as the ganglion cells. The first-order units in the auditory system are comparatively easier to study; however, interaction mechanisms have, thus far, not been demonstrated in first-order auditory neurons.

At higher levels of the visual system, off-responses are easily obtained. Van Hof (19), and Gutierrez and Berger (8) have studied cortical off-responses to photic stimuli. Unlike our off-responses to noise bursts, these responses are present, even in cats that are under deep barbiturate anesthesia. Perhaps, these cortical off-responses simply reflect the fact that visual off-responses seem to be retinal in origin; the retina, of course, contains several orders of neurons. The question of which levels in the nervous system generate off-responses thus may not have a single answer;

it may depend upon the particular off-response that is being studied.

Jung has classified single units in the cat's visual cortex according to their responses to on, off, and steady illumination (11). This suggests the interesting possibility that, even for one sensory system, many different types of off-response may be generated in different ways and at different levels of the nervous system. It becomes clear that off-responses cannot be assumed to have a common basis *per se*. Although the off-responses that have been most intensively studied all appear to involve neural interaction in their production, there always exists the possibility that off-responses can be generated in receptors themselves. There is some suggestion that such a situation may exist in the Octopus eye (9), or in olfactory epithelium (18), where the receptors may be the first-order neurons themselves. If such is the case, even the statement that off-responses always involve neural interaction will have to be changed.

SUMMARY

Auditory off-responses that are abolished by barbiturate anesthesia have been found in the cortex of unanesthetized cats. From the nature of the stimuli used to evoke these responses (bursts of noise), it is certain that they are not the result of click-like transients. It is suggested that these responses represent a release of inhibition because a synchronous burst of activity cannot be found at the periphery when noise is turned off.

ACKNOWLEDGMENTS

We wish to express our gratitude to Professor W. A. Rosenblith, of Massachusetts Institute of Technology, for his generous help and advice during these studies. Mr. R. M. Brown, among the many staff members of the Research Laboratory of Electronics, M. I. T., who have contributed to the execution of these experiments, deserves special mention.

After this manuscript was completed, an article appeared which demonstrates a response found in human brains which we believe to be closely related to the off-responses discussed in this paper. We should like to call attention to this article, as it reports responses appearing at the end of train of clicks which are labile with respect to anesthesia. Reference: Chatrian, G. E., Petersen, M. C., and Lazarte, J. A. Responses to clicks from the human brain: some depth electrographic observations. *EEG clin. Neurophysiol.*, *12*: 479-489, 1960.

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RECRUITING RESPONSES IN THE BRAIN STEM RETICULAR FORMATION [1]

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INTRODUCTION

In 1942, Dempsey and Morison (7, 8) demonstrated the existence of a thalamic system of diffuse cortical projection which is responsible for the production of spindle activity. In 1949, Moruzzi and Magoun (17) established that the cortical desynchronization pattern is dependent upon the activity of an ascending activating reticular system of the brain stem. These pioneers' works were the basis of our progressive understanding of the cerebral processes underlying the behavioral phenomenon of wakefulness. It is now commonly accepted that the bulbo-mesencephalic reticular formation of Moruzzi and Magoun controls the thalamic unspecific nuclei of Dempsey and Morison in such a way as to suppress or release the so-called spindle activity, and, in general, there is indeed a good correlation between the absence or presence of cortical spindling waves and, respectively, the states of attentive activity or mental rest.

During the last years, we have been interested in testing the hypothesis of a reverse action, namely the eventual influence of the thalamic unspecific centers on the reticular structures more caudally situated. Some data on this problem have already been published (20, 21, 22). The present report will describe some new results and gather the overall information, presently available, in order to discuss the functioning of the thalamo-reticular circuits. These experiments concern the study of recruiting responses, elicited by electrical stimulation of the intralaminar or midline nuclei of the thalamus and recorded in the brain stem reticular formation. Such responses have been only incidentally observed in the reticular formation itself (6, 9) or in neighbouring structures (25). On the other hand, the problem of intrinsic interactions in the brain stem reticular structures has been approached on completely different methodological ground by Adey, Segundo and Livingston (1).

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[1] This research has been sponsored by the Air Research and Development Command, United States Air Force, under contract AF 61 (052)-22.

Methods

Sixty eight cats were used in these investigations. The animals were first anesthetized with ether for the surgical preparation. Then they were paralyzed with gallamine [1] or, occasionally decamethonium chloride, and artificial respiration was started. In several experiments, a spinal section at the C_1 level was performed so that no curarising agent was further required; this preparation is referred to in the text as " encephale isolé ".

Various types of electrodes were used: *a*) for the cortex, bipolar silver electrodes separated by about 5 mm, *b*) fort thalamic stimulation, concentric electrodes or pairs of insulated wires enclosed in an injection needle so that the tips appeared at the extremity and were about 0.5 mm apart, *c*) for depth recording, concentric or bipolar electrodes as used foı the thalamic stimulation, also pairs of insulated wires separated by 0.5 to 3 mm or microelectrodes. Glass microelectrodes (NaCl or KCl filled, 5-20 μ at the tip) and stainless steel microelectrodes (10-30 μ at the tip) were used for extracellular unit recording. Intracranial electrodes were stereotaxically oriented with reference to the coordinates of the atlas of Jasper and Ajmone-Marsan (13). The potentials were led through ordinary channels including cathode followers and RC coupled preamplifiers, and recorded on a two or four-beam cathode ray oscilloscope. Long-time sequences of spontaneous unit activity were also fed into a magnetic tape-recorder. In addition, an inkwriter was used as a monitoring device for direct observation of the smooth wave activity.

The technical procedures were different for the various experiments which are to be reported here. Thus relevant details will be given in the text or legends of the figures, in relation with the results of each investigation.

Results

1. *General description and evidence of recruiting responses in the mesencephalic region.* — With implanted glass and metal microelectrodes, concentric and dual coarse electrodes, responses have been recorded in the central core of the mesencephalon, which were closely similar to the well known cortical responses evoked by low frequency stimulation of certain thalamic areas. The reasons for identifying these potentials as a typical " recruitment " have

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[1] Flaxedil (gallamine) was kindly supplied by the Société SPECIA, Paris.

already been discussed (20, 21) and therefore, will be only briefly summarized here.

1) A repetitive stimulation of the thalamic intralaminar or midline regions generated, in the mesencephalon, a succession of potential deflections of increasing amplitude. There was no response or only a very small wave following the first shock. The maximum amplitude was reached after the third to the sixth stimulus (Fig. 1).

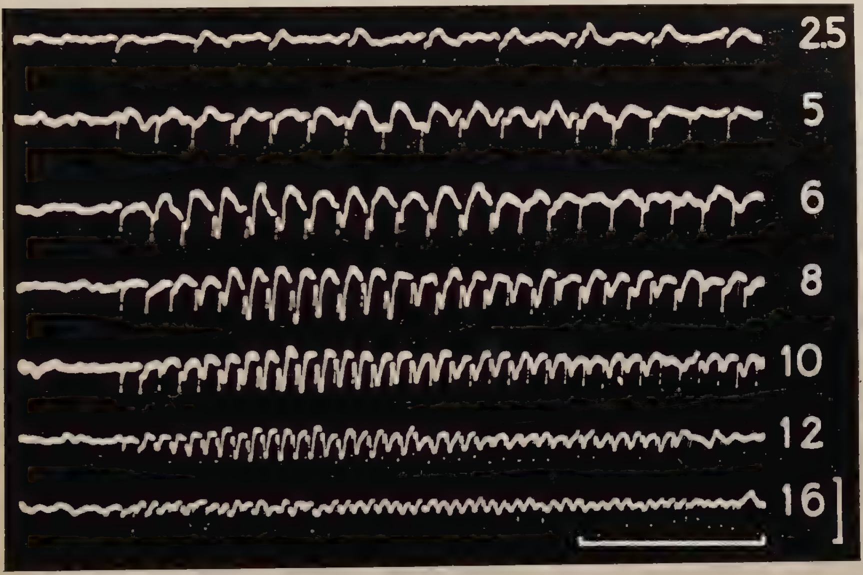


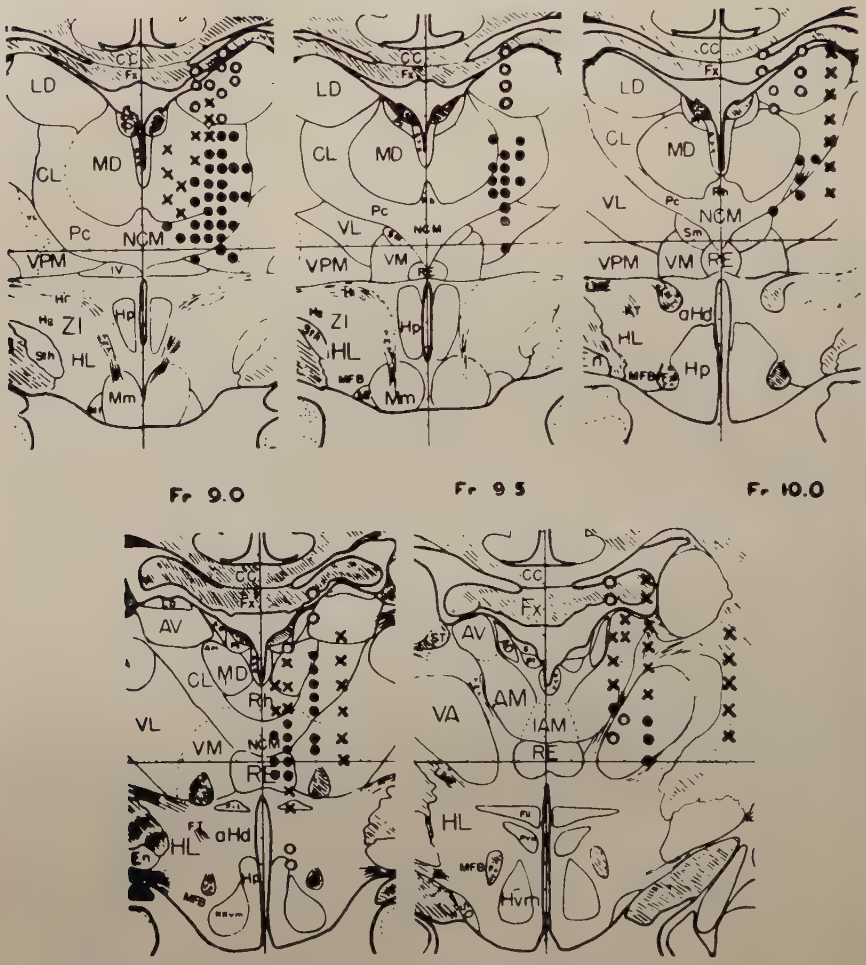
Fig. 1. – *Influence of the frequency of stimulation of a thalamic nucleus on the recruiting responses recorded in the mesencephalon.*

Cat immobilized with Flaxedil. Bipolar recording from the medial mesencephalon under the level of the central gray matter. Stimulation of N. centrolateralis of the thalamus (2 V, 1 msec, frequencies per second are indicated at the right of the traces). True recruitment appeared for frequencies between 6 and 12/sec. Time calibration: 1 sec. Voltage calibration: 100 μV. Reproduced from Schlag (21), by permission of the editor.

2) Later on, in spite of the continuation of the repetitive stimulation, the responses markedly decreased and frequently (but not consistently) there was a cyclical alternation of higher and smaller potentials (waxing and waning).

3) The latency of the responses was between 20 and 50 msec.

4) A typical spindle organization of the potentials could be obtained only for optimum frequencies (6 to 12/sec) of stimulation (Fig. 1).



Fr 9.0 Fr 9.5 Fr 10.0

Fr 11.0 Fr 12.0

Fig. 2. – *Map of thalamic sites yielding recruiting responses in the midbrain reticular formation.*

Drawings reproduced from the stereotaxis atlas of the diencephalon of the cat (13). Black dots: sites of stimulation which induced mesencephalic recruiting responses. Circles: sites of stimulation which induced other types of repetitive responses in the mesencephalon. Crosses: sites of stimulation which did not give any response in the mesencephalon. These locations are only approximative. Most of them correspond to several experiments performed on different animals.

5) The sites of stimulation, causing such responses in the mesencephalon, corresponded to the region of the midline, intralaminar and ventral anterior thalamic nuclei. The main locations which were tested in these experiments and led to the production of typical recruitment, are indicated by black dots in Fig. 2.

2. *Evidence of recording recruiting responses from reticular structures.* — The responses described in the preceding section were commonly recorded from a ponto-mesencephalic area localized at the Horsley-Clarke frontal planes +1 to +3, under the level of the central gray matter. The question arose to know whether the potentials actually had their origin in this region or originated somewhere else in the vicinity and spread by pure electrical conduction in a volume conductor. Actually, as shown in Fig. 3, the amplitude of the recruiting waves was higher (about 4 to 10 times higher) when they were recorded with monopolar than with bipolar electrodes. In this last case, the potential deflections were sometimes quite small (see trace 2 in lower record), probably because the bipolar electrodes which were close together (about 1 mm) simultaneously captured in-phase signals and only displayed the minor differences of voltage in the synchronized activity at the two sites of recording. According to this view, we must assume, in comparing the monopolar and bipolar reticular recordings of Fig. 3, that the recruiting potentials affected *en masse* the medial part of the mesencephalon. More laterally, somewhat larger potentials were found (trace 4 in lower record) so as to suggest, at that place, a less homogeneous distribution of the potentials. A similar analysis was made along a vertical axis with concentric electrodes introduced 2 mm lateral in the mesencephalon. Successive recordings of recruiting responses indicated that the largest potentials were obtained at the horizontal levels 0 to –2, probably when the electrodes entered the reticular formation.

In addition, microelectrode recordings demonstrated that recruiting potentials might be obtained in the surrounding field of bulbar as well as mesencephalic cells responding with a long latency to a large variety of peripheral stimuli. As fully described in Section 6, the unitary activity of these reticular neurons was strikingly altered by the volleys of impulses originating in the unspecific thalamic nuclei.

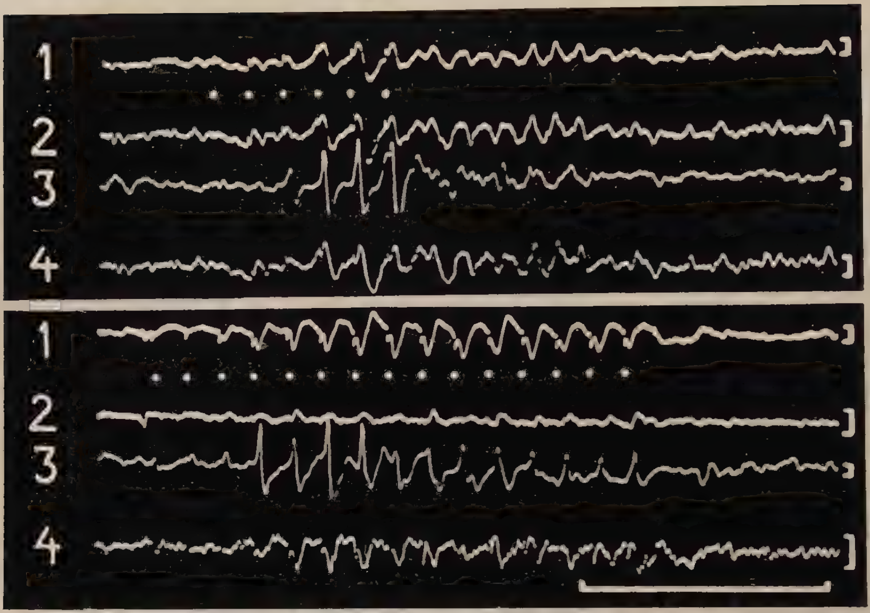


Fig. 3. – *Comparison of monopolar and bipolar recordings of recruiting responses in the mesencephalic reticular formation.*

Cat immobilized with Flaxedil. Stimulation of N. paracentralis of the thalamus (1.75 V, 1 msec, 7.5/sec). Shocks indicated by dots. Recordings in the mesencephalon, under the level of the central gray matter were made with three insulated wires placed on a frontal plane at 2, 3 and 4 mm of the midline (they are called respectively internal, median and external electrodes).

Upper record. 1, 2 and 4 are respectively the reticular monopolar recordings with the internal, median and external electrodes. 3: bipolar recording from the homolateral gyrus marginalis of the cortex.

Lower record. 1: monopolar recording with the reticular internal electrode. 2 and 4: reticular bipolar recordings made respectively with the internal and median and with the median and external electrodes. 3: bipolar recording from the homolateral gyrus marginalis of the cortex. Note the difference in size of the recruiting responses in the bipolar recordings 2 and 4.

Time calibration: 1 sec. Voltage calibration: 50 μV.

3. *Other repetitive responses in the reticular formation.* — We have restricted the use of the denomination " recruiting responses " to the pattern defined in Section 1, according to the criteria proposed by Jasper and Ajmone-Marsan (12). However it must be pointed out that several other kinds of repetitive potentials may be evoked in the reticular formation, especially by appropriate stimulation of structures in the vicinity of the diffusely projecting thalamic nuclei.

Such responses were usually obtained for instance when the stimulating electrode was located in the fornix or in the upper thalamus close to the stria medullaris. In these cases, the potentials recorded in the mesencephalon corresponded to the descriptions given by Adey, Sunderland and Dunlop (2), when they stimulate at the same places. Other responses were induced by subthalamic stimulation. The principal location of the sites yielding non-recruiting repetitive potentials in the brain stem are indicated by circles in Fig. 2. These non-recruiting potentials were readily distinguished from recruiting ones by several features, including latency, presence of a response after the first shock, progressive and continuous increase in amplitude and convulsive after-activity.

On the other hand, the iterative excitation of most thalamic nuclei did not evoke any response in mesencephalic reticular structures (negative results obtained from the points marked in Fig. 2), although it sometimes gave raise to well developped cortical potentials.

4. *Influence of the cerebral cortex on the reticular recruitment.* — Cortical influences on the brain stem reticular formation have been demonstrated long ago. For instance, Bremer and Terzuolo (4, 5) induced a generalized desynchronisation of the cerebral cortex by cortical stimulation and they showed that this effect was mediated by the ascending activating reticular system. Similarly Hugelin and Bonvallet (10, 11) suggested the existence of a cortico-reticulo-cortical circuit, although they considered this system as a negative feedback mechanism in opposition to the former authors. Formal evidence of cortical projections on reticular structures has already been presented (9) and, particularly, it has been shown that axonal discharges, synchronized with the cortical recruiting potentials may be conducted by the pyramidal fibers (3). Thus, it seemed interesting to analyze the role of the cerebral cortex in the induction or transmission of the recruitment recorded in reticular structures.

i) Consistently, the appearance of recruiting responses in the reticular formation, whatever site of thalamic stimulation was selected, was always paralleled by the simultaneous development of cortical recruitment. Although this rule did not suffer any exception in the whole series of experiments, sometimes the reticular and cortical recordings differed largely in the rate of the amplitude increase and the time when a maximal deflection was reached. But in those cases, always the cortical recruiting was lagging. This cortical recruitment was also much less stable and the successive waves were not so regular. The cortical process thus appeared to be an indirect response driven by structures different from those underlying the recording electrodes.

ii) Actually, the unspecific thalamic nuclei are the very origin of recruiting phenomena, whatever the place where the potentials are recorded. Nevertheless a low-frequency repetitive stimulation of other structures, including the cortex itself, may also be found effective, probably by an indirect activation of thalamic neurons (27). Thus attempts were made to elicit repetitive responses in the mesencephalic reticular formation by electrical stimulation of the cortex. Shocks were applied by bipolar surface electrodes at a rate of 6 or 10/sec and with various intensities. Small responses in the mesencephalic region were obtained in this way, but only in rare instances when the rostral part of the homolateral frontal gyrus was stimulated. Their amplitude was very weak, even for considerable intensities of current, which reached the threshold for convulsions. They might be supposed to be a recruitment process, because they developped synchronously with true cortical recruiting potentials.

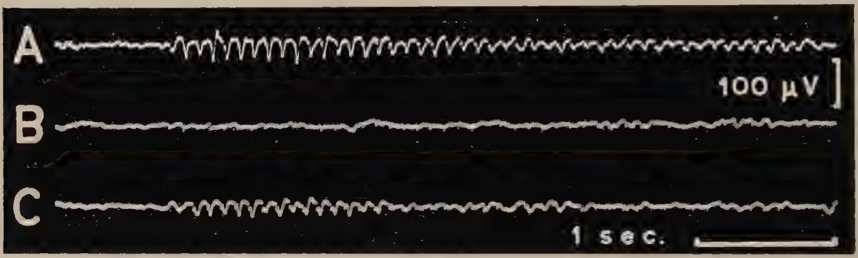


Fig. 4. – *Lasting influence of a cortical stimulation on subsequent recruiting responses in the mesencephalon.*

Cat immobilized with Flaxedil. Stimulation of N. paracentralis of the thalamus (1.75 V, 1 msec, 10/sec). Bipolar recording in the mesencephalon at the border of the central gray matter. A: control. *B*: 2 min after an intense cortical stimulation (frontal cortex, 12.5 V, 0.5 msec, 100/sec) which induced hypersynchronous cortical waves for about 1 min and a dilatation of the pupils, the EEG was desynchronized again at the time of recording. C: 6 min after the cortical stimulation, when the recruiting responses reappeared. Time calibration: 1 sec. Voltage calibration: 100 µV.

iii) Changing the frequency of cortical stimulation from low values to 100-200/sec was expected to have the opposite effect, namely to inhibit the recruitment when the later was induced by thalamic shocks, and cause a widespread desynchronization. This result was obtained while recording from the mesencephalic region. It was found that a 100/sec train of non convulsive shocks could suppress the recruiting responses for a period which largely outlasted the duration of the stimulation. An inhibiting effect of 3 or 5 minutes might even be obtained when using cortical stimuli which reached the threshold for a short burst of cortical hypersynchronous convulsive waves (Fig. 4).

iv) Exclusion of the cerebral cortex, on the other hand, did not suppress the mesencephalic recruitment. In three surgically decorticate cats, occurrence of typical recruiting responses in the reticular formation was observed after the ablation (Fig. 5 *B* and *C*). In two other animals, the hemispheres were largely exposed, the dura-mater was removed and the thalamus was stimulated at regular intervals while cooling the cerebral cortex with CO_2 dry ice. This procedure reduced midbrain recruitment only after 2 minutes but did not abolish it even after 4 minutes of cortical cooling (Fig. 5 *A*). The late depression may be explained by assuming that refrigeration had reached subcortical structures.

5. *Pathway from the thalamus to the reticular formation.* — We have demonstrated in the preceding section that the cerebral cortex is not strictly necessary for eliciting recruiting potentials in the mesencephalon. This result suggests that the whole or most important pathway runs subcortically.

In order to determine more accurately the location of the thalamo-reticular connections, we studied systematically the effects of circumscribed lesions made in a diencephalic frontal plane separating the stimulating from the recording electrodes. A 8 to 10/sec stimulation of the nucleus centro-lateralis gave rise to typical potentials, which were recorded homolaterally on the cortex and at the ponto-mesencephalic junction underneath the central gray matter (Fig. 6 *A*, upper record). An array of 6 electrodes, spaced from each other by 1.5 mm, was then inserted in the brain at the Horsley-Clarke frontal plane + 6.5 (see section of the brain in Fig. 6). A constant current of 3 mA was allowed to pass for periods of 15 sec between the uninsulated tips of adjacent pairs of

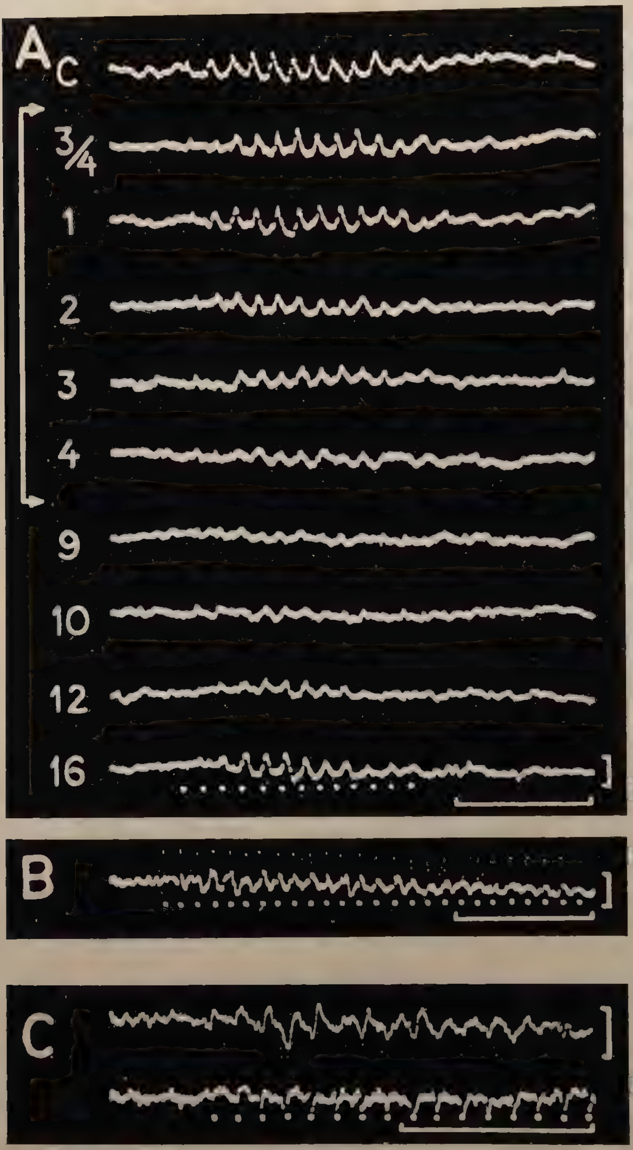


Fig. 5. – *Influence of surgical and functional ablation of the cerebral cortex on the mesencephalic recruitment.*

A: cat immobilized with Flaxedil. Stimulation of N. centrolateralis of the thalamus (7 V, 0.5 msec, 8/sec). Bipolar recording with a concentric electrode from the mesencephalon, under the level of the central gray matter. The upper trace is the control (C). Immediately after this recording, the cortex, largely exposed, was cooled with CO_2 dry ice during 5 min (period indicated by the left cortical bar). Records were taken periodically as indicated in minutes at the left of each trace. The recruiting responses were abolished only about 4 min after the termination of the cortical cooling (at 9 min). Shocks indicated by dots. Time calibration: 1 sec. Voltage calibration: 100 µV.

B: decorticate cat. Stimulation of N. paracentralis of the thalamus 4 V, 1 msec, 10/sec). Bipolar recording from the mesencephalon, 1h and 20 min after the decortication. Shocks indicated by dots. Time calibration: 1 sec. Voltage calibration: 100 µV.

C: decorticate cat. Stimulation of N. paracentralis of the thalamus (4 V, 1 msec, 7.5/sec). Monopolar recording from the mesencephalon. Upper trace: control. Lower trace: after section with a 5 mm broad spatula introduced vertically at the frontal plane 6 homolaterally close to the midline. Note the disappearance of the responses. Shocks indicated by dots. Time calibration: 1 sec. Voltage calibration: 100 µV.

electrodes. Hence under stereotaxic control, a progressive coagulation could be performed by successive steps of 1 mm depth in a 10 mm broad area extending bilaterally in the thalamus. Following each phase of coagulation, the recruiting response in the mesencephalon was evoked and tested. The effects of lesions placed at various

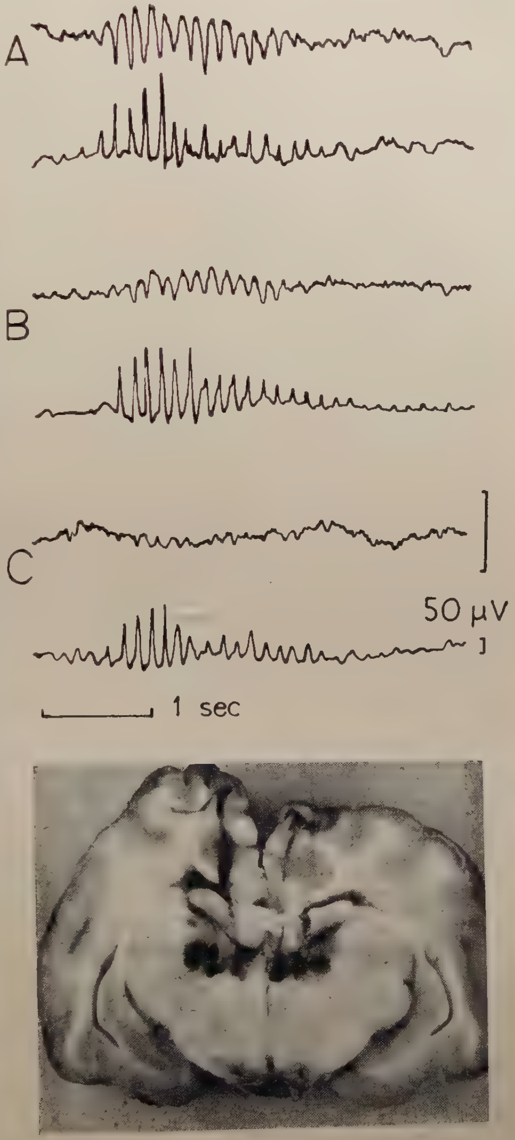


Fig. 6. – *Pathway of conduction of recruiting impulses toward the reticular structures of the brain stem.*

Cat immobilized with Flaxedil. Stimulation of N. centromedialis of the thalamus (4 V, 1 msec, 7/sec). Upper traces: from the mesencephalon; lower traces: from the homolateral gyrus marginalis of the cortex. *A*: control, recruiting responses at their full amplitude in both leads. *B*: after bilateral coagulation at the horizontal level H + 3, as shown on the section. C: after a further coagulation 1 mm down on the right (homolateral) side, recruiting responses disappeared in the mesencephalon. The lesions may be seen on the brain section; they were made by 6 electrodes on a frontal plane. Time calibration: 1 sec. Voltage calibration: 50 µV.

sites were studied in that way and, in four animals, we observed that the more critical or practically complete disappearance of the mesencephalic responses was dependent upon a bilateral coagulation corresponding mostly to the upper border of the centre median and to the region of the posterior commissure (Fig. 6 *B*). When the coagulation was carried 1 mm deeper on the homolateral side, the decrease of the mesencephalic responses was still more evident (Fig. 6 *C*). This was interpreted as suggesting a bilaterality of the pathway with a predominant homolateral component. The cortical recruitment which was used as a control was not seriously affected (lower trace). As another consequence of the coagulation in that region, slow waves and spindle bursts appeared suddently more frequent on the cortex, suggesting that ascending and descending impulses in the thalamo-reticular pathways were simultaneously affected by the coagulation procedure.

A section with a 5 mm broad spatula was also performed in the same region, homolaterally, in a decorticate preparation and similar results were observed: the reticular recruiting responses disappeared, as shown in Fig. 5 *C*.

6. *Unit analysis.* — Li, Cullen and Jasper (15), Jung, Creutzfeldt and Baumgartner (14) gave a full account of the unit activity they recorded from cortical neurons under conditions of recruitment. The behavior of ponto-mesencephalic and bulbar reticular nerve-cells under the same circumstances appeared much less stereotyped. We do not think that the lack of homogeneity in this case could be entirely accounted for by the differences in the regional structures we recorded from, because any combination of the typical features listed hereunder could be observed from a few cells lying in close vicinity. Sometimes several units were recorded simultaneously (occasionally with the same microelectrodes) and they disclosed very distinct – eventually opposed – types of responses (for instance see Fig. 8 *C*). In addition, we did not find any evidence of relation between the characteristics of the responses and the site of the stimulation, provided the latter was able to generate true recruiting potentials.

In the following description, we called reticular the units which fulfilled one of the listed requirements: *a*) responding to more than one kind of stimulation, either tactile, proprioceptive, auditory or visual, *b*) responding only to somesthetic impulses arising in a large peripheral receptive area, *c*) non responding but being closely surrounded by other units having the properties *a*) or *b*). On theoretical grounds, we considered as unjustified to disregard units *c*) which may have their own important functional significance (16, 18, 21, 23).

i) *One or two spikes responses.* Some units, practically silent in the absence of experimental stimuli, discharged one (Fig. 7 *A*) or two spikes (Fig. 7 *B*) when solicited by the thalamic repetitive

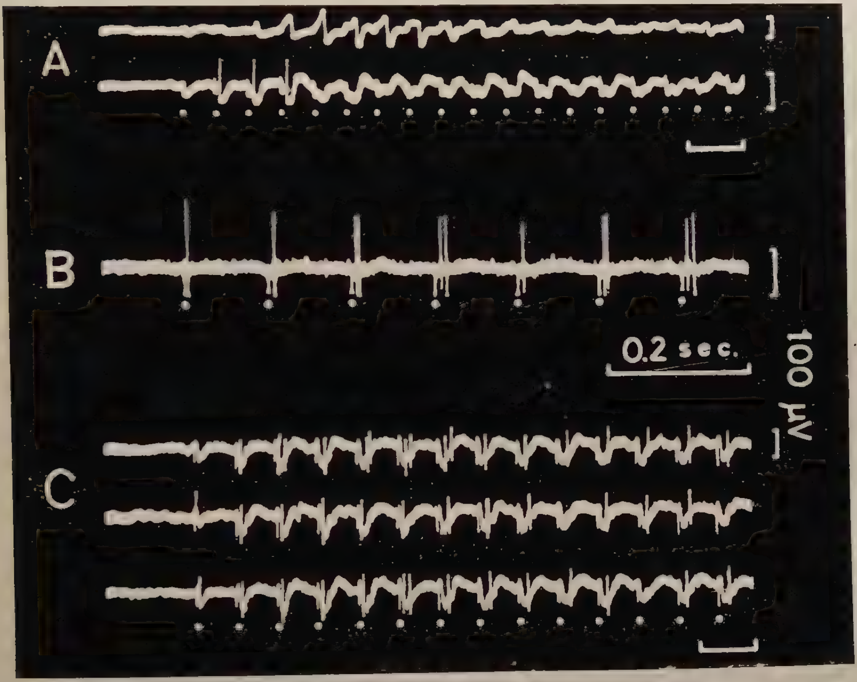


Fig. 7. – *Reticular unit responses to the stimulation of an intralaminar nucleus of the thalamus.*

A: encephale isolé, stimulation of N. paracentralis of the thalamus (4 V, 0.5 msec, 10/sec). Upper trace: from the gyrus marginalis; lower trace: microelectrode recording from the mesencephalon.

B and *C*: cat immobilized by Flaxedil, stimulation of N. paracentralis of the thalamus (5 V, 0.5 msec, 7.5/sec). *B*: short time constant microelectrode recording from a bulbar unit (also responding to the mechanical stimulation of the four limbs). *C*: responses of a mesencephalic unit (responding to mechanical stimulation of the caudal part of the body): three successive trains of thalamic stimuli, the latency of the responses increased in each case.

Time calibrations: 0.2 sec. Voltage calibrations for low frequency waves (except in *B*): 100 µV. Shocks artefacts indicated by dots. In these and following records of unitary discharges, negativity is upward.

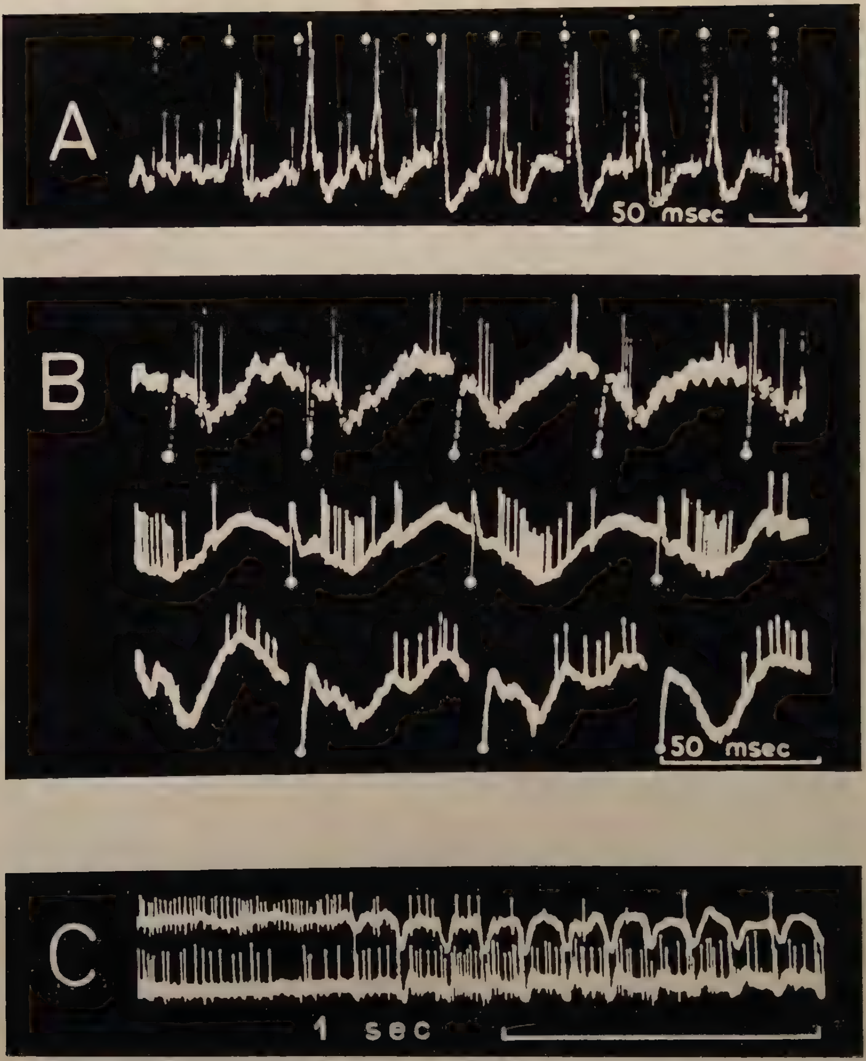


Fig. 8. – *Reticular unit responses to the stimulation of an intralaminar nucleus of the thalamus.*

Cats immobilized with Flaxedil. *A*: stimulation of N. centrolateralis of the thalamus (5 V, 0.5 msec, 7.5/sec), microelectrode recording from the ventral mesencephalon (unit responding to the mechanical stimulation of the whole heterolateral hindpaw). *B*: stimulation of N. centrolateralis of the thalamus. Microelectrode recordings from mesencephalic units in three different experiments (upper unit responding to mechanical stimuli everywhere on the body and to auditory stimuli; unit of the second trace, responding to the mechanical stimulation of the heterolateral hindpaw and the back; unit of the third trace, responsiveness not tested). Time volleys. These unit responses usually appeared with the second or third shock, when the recruiting potentials were just building up. The unit firing could be correlated, however not completely, with the amplitude of the smooth waves. In general, the discharges appeared more easily at the beginning of the train (Fig. 7 *A*), even when the recruiting potentials remained of the some amplitude. The latency of the spikes was about 20 to 50 msec. Sometimes the latency was found to increase progressively with the repetition of the shocks (Fig. 7 *C*). Units of this kind were observed in both mesencephalic and bulbar regions.

ii) *Short latency responses.* In two cases out of more than 150 observations, short latency responses (10 msec) were recorded (Fig. 8 *A*). The discharges occurred at the crest of an initial early negative potential.

iii) *Modulation.* The most commonly encountered response consisted in a modulation of the naturally sustained spontaneous activity, in relation with the baseline waves. In this case, the grouping of the spikes appeared most evident from the third to the tenth shock. The grouping did not occur systematically in the same phase of the wave cycle but varied in its position from cell to cell (Fig. 8 *B*), although it was perfectly constant and reproducible for any given cell. Among the possibilities which were commonly encountered, the bursts were coincident either with the negative crest (third trace), the positive wave (second trace) or both flanks, negative-positive (first trace) and positive-negative of the waves. Frequently, the modulation resulted in an evident increase of the background activity as considered in the next paragraph.

iv) *Activation and inhibition.* Activation without modulation was a rather rare feature, of which Fig. 8 *C* gives an illustration. In this experiment, we simultaneously observed an accelerated mesencephalic unit (lower trace) and an inhibited bulbar unit (upper trace), under the influence of a thalamic stimulation. The phenomenon of inhibition deserves further attention because its occurrence during the stimulation was mostly observed for cells recorded in the lower part of the reticular formation. In rare instances was it

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calibrations: 50 msec in *A* and *B*. Shocks indicated by dots. *C*: stimulation of N. paracentralis of the thalamus (5 V, 0.5 msec, 7.5/sec). Upper trace: bulbar unit (responding to the mechanical stimulation of the four paws); lower trace, mesencephalic unit (responding to the mechanical stimulation of the caudal part of the body). Time calibration: 1 sec.

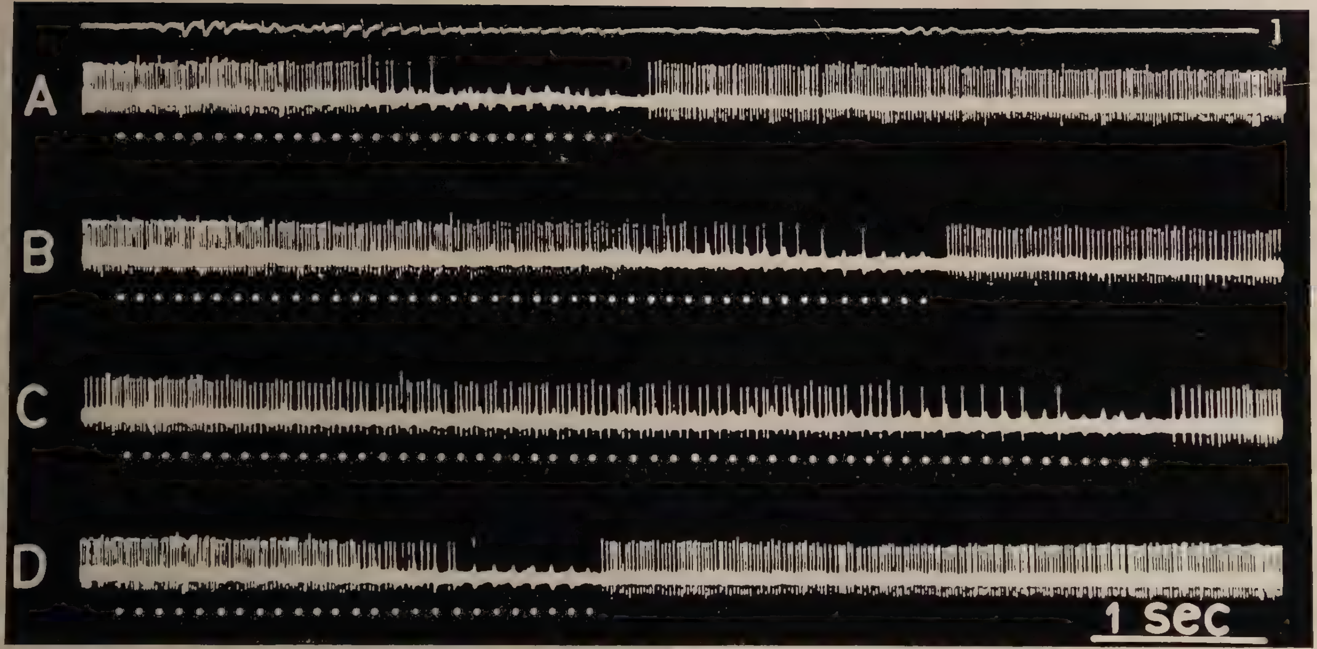

Fig. 9. – *Inhibition of a bulbar unit, elicited by stimulation of an unspecific thalamic nucleus.*

Cat immobilized by Flaxedil. Stimulation of the N. ventrolateralis of the thalamus (10 V, 1 msec, 9/sec, shocks indicated by dots). Microelectrode recording from a bulbar unit (responding to the mechanical stimulation of the hindpaws and caudal part of the body). These records were taken with a short time constant in order to suppress the baseline fluctuations. Simultaneous recording of the cortical recruitment on the homolateral gyrus marginalis may be seen in *A* (voltage calibration for this record: 250 µV). A 10 sec interval elapsed between *A* and *B*, *B* and *C*: note progressively increasing delay of the inhibitory effect. In record *D*, taken 60 sec after *C*, the delay of the inhibitory response has come back to control values. Time calibration: 1 sec.

noticed in the ponto-mesencephalic region (Fig. 12). Such inhibition seems to be an active process, since its onset may be delayed by applying at short intervals the recruiting stimulations (Fig. 9).

v) *Post-excitatory extinction.* It seems reasonable to distinguish from the above described inhibition, the phenomenon of post-excitatory extinction, which was commonly encountered when recording from mesencephalic as well as bulbar neurons. In this case, the silent pause never appeared before the last shock. It was quite similar to the effect of a positive after-potential and, in fact, its duration was clearly dependent upon the length of the thalamic stimulation (Fig. 10). Inhibition has already been obtained from cortical neurons following a single thalamic shock (14) but we cannot appreciate the similarity of this observation with our own mesencephalic recordings.

vi) *No response.* We did not make any statistical computation of the unresponding cells of the mesencephalic and bulbar reticular formation, because in too many experiments we dropped them deliberately from our files. We can roughly estimate that less than half of our units did not respond to a recruiting stimulation of the thalamus.

7. *Convergence of sensory and recruiting impulses on reticular neurons.* — In order to determine whether or not the recruitment might alter the responsiveness of reticular neurons receiving impulses from the peripheral sensory afferences, the effects of convergence were observed during microelectrode recording in the mesencephalon. This analysis is not yet fully complete. However the results already obtained under definite conditions of convergence were sufficiently consistent to appreciate tentatively the extent of possible interactions. We shall only report on two experimental situations.

Thalamic stimuli had to be applied repetitively at a 6 to 12/sec rate in order to build up the typical recruiting potentials in the mesencephalon. In the first case, skin electrodes were located in the peripheral receptive field of the neuron under study and shocks were delivered simultaneously at the periphery and in the diencephalon.

In the experiment illustrated by Fig. 11, the spontaneous discharges of a mesencephalic reticular cell were modulated by the largest recruiting potentials (lower trace in *A* and *D*). On the other

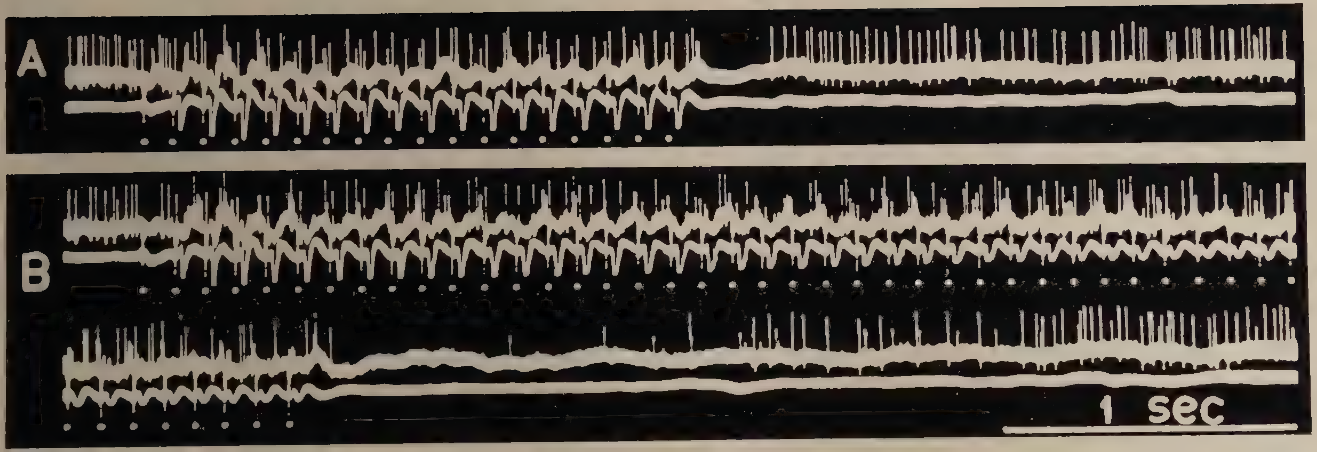

Fig. 10. – *Influence of the duration of the recruiting stimulation on the post-excitatory extinction of a mesencephalic unit.*

Cat immobilized with Flaxedil. Upper trace: microelectrode recording from a mesencephalic unit (responding to the mechanical stimulation of the hindpaws); lower trace: from the cerebral cortex. Stimulation of N. centrolateralis of the thalamus (10 V, 1 msec, 9.5/sec, shocks indicated by dots). *A*: the duration of the stimulation was 1.9 sec, the inhibition lasted 0.25 sec. *B*: the duration of the stimulation was 4.6 sec, the inhibition lasted more than 1 sec. Time calibration: 1 sec.

hand, repetitive peripheral shocks induced and maintained a clear-cut acceleration of the spontaneous firing (lower trace in *B*). When both stimulations were carried together, the effects of the peripheral volleys were found to be dominant: recruitment was inhibited on the cortex as well as in the reticular formation, the modulation effect was more or less cancelled, whereas the increased firing caused by the sensory impulses remainded unaltered (lower trace in *C*).

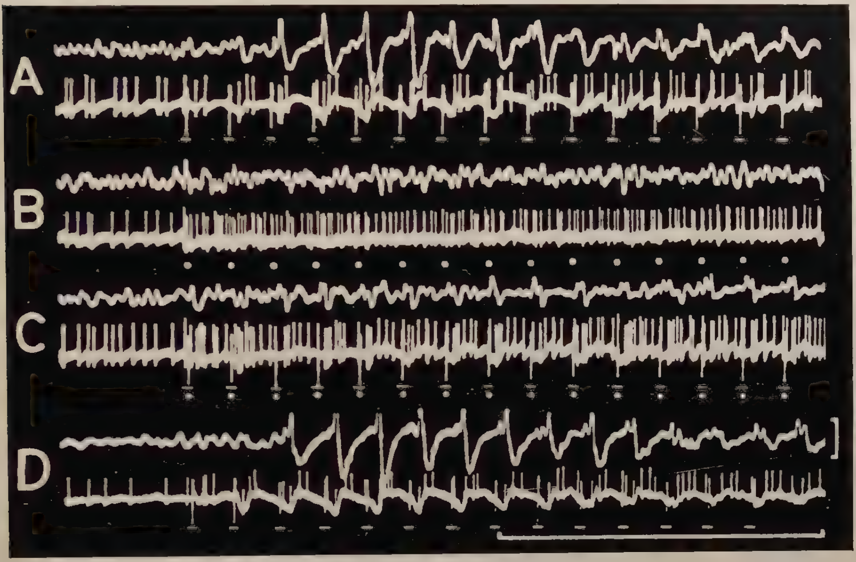

Fig. 11. – *Convergence of thalamic and somesthetic impulses on a mesencephalic reticular neuron.*

Cat immobilized with Flaxedil. Upper traces from the gyrus marginalis; lower traces, microelectrode recording from a mesencephalic unit (responding to the mechanical stimulation of the four paws). *A*: stimulation of ventromedian thalamic nucleus (5 V, 1 msec, 7.5/sec), the thalamic shocks are indicated by horizontal bars. The discharges occurred mainly on the positive-negative flanks of the higher baseline potentials. *B*: stimulation of the heterolateral hindpaw (17.5 V, 1 msec, 7.5/sec, shocks indicated by dots). *C*: simultaneous thalamic and somesthetic stimulations under the same conditions as in *A* and *B*, the cortical and mesencephalic recruitments were decreased, the unit activity was enhanced as in *B*. *D*: final control of recruiting responses under same conditions as in *A*. Time calibration: 1 sec. Voltage calibration for the cortical records: 200 μV.

In another set of experiments, we used a single peripheral shock, which was adjusted just above the threshold for a reticular unit. By limiting the strength of the peripheral stimulation, we intended to balance the convergent afferent impulses and reveal an eventual interaction between them. The experiment presented in Fig. 12 was selected because it happened that the spontaneous activity of the reticular neuron was inhibited during the recruitment in this case (traces *C* and *D*). The response to a single peripheral stimulus may be seen in *A* and *B*. It consisted of an early high-frequency burst of discharges followed by a silent period and finally a rebound of the activity. This response was almost unaltered when the peripheral shock was timed in order to appear either at the beginning (trace *E*) or later in the course of the recruiting process (trace *F*). Practically the inhibitory effect of the thalamic volleys was not sufficient as to preclude the sensory response.

Thus far, we found no evidence of a possible alteration of reticular responses to peripheral stimulation under conditions of recruitment, even in the favorable case where the peripheral stimulus is weak enough as to keep the recruitment itself unaffected (Fig. 12). A further analysis of other experimental conditions is however required in order to generalize this conclusion.

8. *Evoked recruiting responses, related after-activity and spontaneous spindle bursts in the mesencephalic reticular formation.* — The similarity between recruiting responses and spindles has been noted very early by Dempsey and Morison (8). Later on, various studies established that these evoked and spontaneous patterns had the same thalamic origin and were practically equivalent. Therefore, it seemed tempting to observe the occurrence of spindle burst activity in the reticular formation, to seek for some peculiar features noticed in the case of the recruiting responses and compare the results with those we reported so far in this paper.

Spontaneous spindle bursts may be observed in the reticular area of the brain stem. They were shown, for instance under barbiturate narcosis, by Bremer and Terzuolo (5). Nevertheless, their occurrence is less frequent than in mesial thalamus and sensorimotor cortex (24), and the amplitude of the waves appears to be much smaller in the recordings from that region than from elsewhere, namely the medial diencephalic nuclei or the cerebral cortex (19). A comparison between monopolar recordings of simultaneous cortical and reticular spindle waves shows that the amplitude of the former is generally about 10 times the amplitude of the latter. This measurement was made with electrodes of the same size (0,5 mm

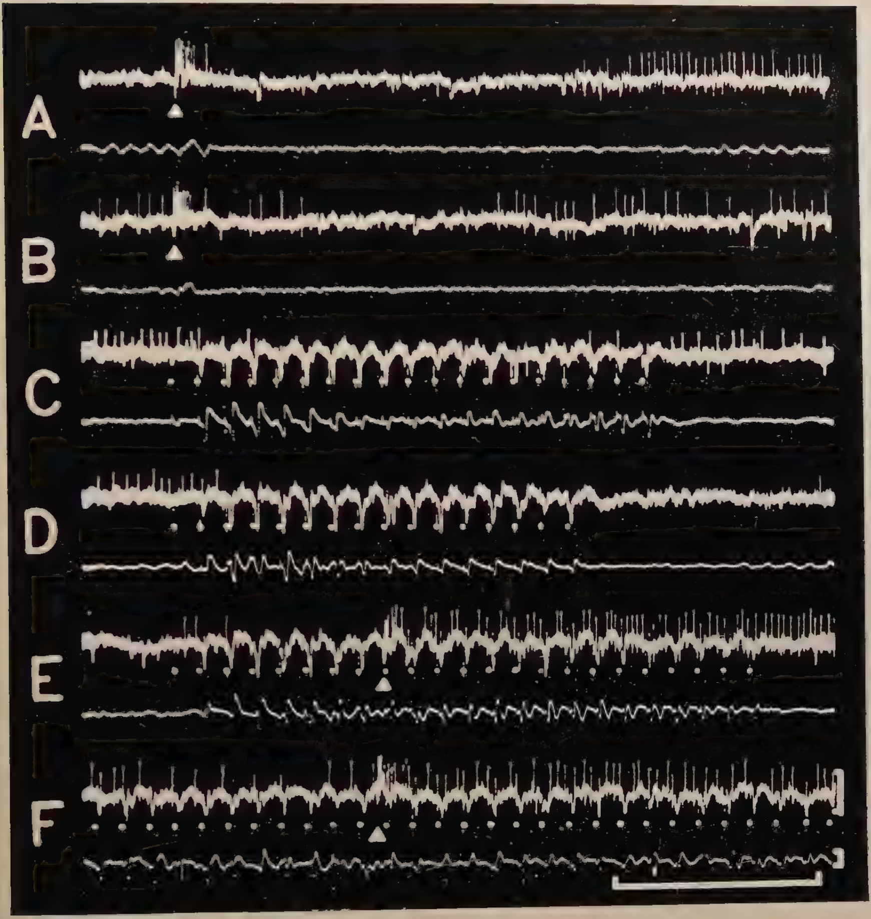

Fig. 12. – *Convergence of thalamic and somesthetic impulses on a mesencephalic neuron.*

Cat immobilized with Flaxedil. Upper traces; microelectrode recording from a mesencephalic unit (responding to the mechanical stimulation of the caudal part of the body). Lower traces: from the gyrus marginalis. *A* and *B*: single shock (6 V, 10 msec, indicated by a triangle) applied on the skin of the lumbar region. *C* and *D*: repetitive stimulation of N. centrolateralis (5 V, 1 msec, 8/sec, shocks indicated by dots). Note the inhibition of the spontaneous firing. *E* and *F*: the peripheral stimulus (triangle) is applied during the thalamic stimulation (dots) at the beginning of the train (*E*) and later in the course of a long train (*F*). Note the presence of the short latency high-frequency burst in response to the sensory impulses and the return of sustained discharges. Time calibration: 1 sec. Voltage calibrations for low-frequency waves: 100 μV.

at the tip) and the value of this ratio is given for the most favorable cases where spindling was relatively evident in the mesencephalic reticular formation. But, most times, there were no slow waves in the mesencephalon simultaneously with the cortical spindle bursts.

An absolute estimation of this kind, of course, could be very inaccurate and difficult to assess, taking in account the variability in the conditions of recording from different structures. So, if by some means, a valuable criterion could be found for a relative evaluation of the potentials, the comparison would be more objective and meaningful. Recruiting responses might constitute such a criterion (Fig. 13 and 14). As far as one admits the analogy of these responses with the spontaneously occurring spindles, one may assume that both kinds of potentials may reach about the same amplitude, as it is actually the case for cortical recordings. Indeed, spindle bursts on the cortex were quite comparable in size to the cortical recruiting responses (Fig. 13, upper traces). But in no instance, the spindle bursts were more than a small fraction (about 1/8, rarely 1/4) of the recruiting responses, when the recording electrodes were in the mesencephalon (Fig. 13, lower traces). Although there are considerable differences related to the position of the

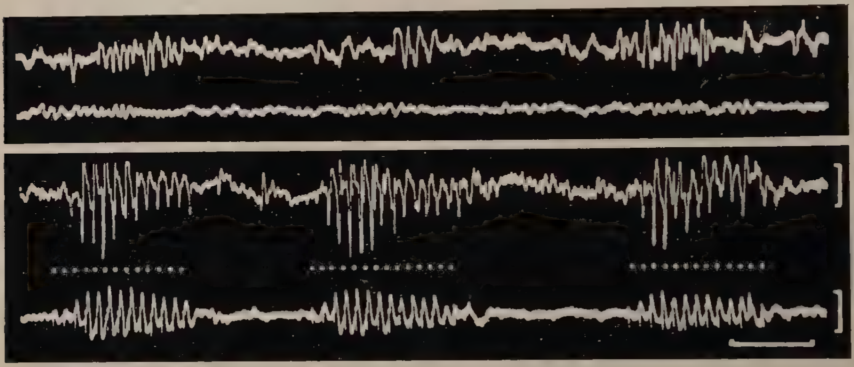

Fig. 13. – *Comparison of spindles and recruiting responses on the cerebral cortex and in the reticular formation of the brain stem.*

Cat immobilized with Flaxedil. In both records, upper trace from the gyrus marginalis (bipolar electrodes), lower trace from the mesencephalic reticular formation (bipolar electrodes).

Upper record: spontaneous activity. Lower record: three trains of thalamic shocks (1.75 V, 1 msec, 7.5/sec) in N. paracentralis (shocks indicated by dots). Time calibration: 1 sec. Voltage calibrations: 100 μV.

electrodes in the mesencephalic reticular formation and to the recording technique (monopolar or bipolar), more often the spindles would be practically unnoticed when using the same gain for both evoked (recruiting) and spontaneous (spindling) potentials.

Besides the fully spontaneous spindle bursts and the fully driven recruiting responses there is the classical observation of after-activity triggered by one or a few thalamic volleys. We have already men-

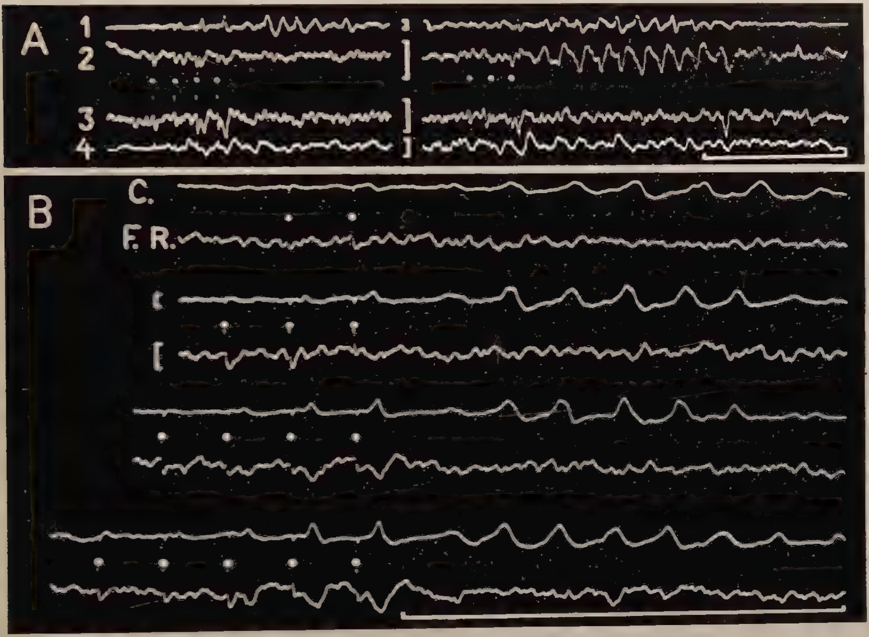

Fig. 14. – *Comparison of recruiting responses and tripped spindling waves in different cortical and subcortical regions.*

Cat immobilized with Flaxedil. Stimulation in N. reuniens of the thalamus (6 V, 1 msec, 8/sec, shocks indicated by dots).

A: four beam cathode ray recordings, with bipolar electrodes, from the lateral gyrus (1), lateral geniculate nucleus (2), mesencephalic reticular formation (3) and superior colliculus (4). All records were homolateral with respect to the sites of stimulation, except that from the geniculate nucleus. Tripped spindles were seen on the cortex and sometimes in the superior colliculus and heterolateral geniculate nucleus (second part of the record) but never in the reticular formation.

B: same experiment with high speed recording to show the details of the recruiting responses on the cortex (*C*) and in the reticular formation (F. R.). The gains were adjusted to display responses of equal size in both traces. Trains of 2, 3, 4 and 5 thalamic shocks were successively tested. Note the regularity of the cortical after-activity and the absence of such an after-activity in the reticular formation.

Time calibration: 1 sec. Voltage calibrations: 100 μV.

tioned the lack of after-activity in the reticular formation following a single shock applied to an unspecific thalamic nucleus (21). Whereas this after-activity, which consisted of cyclical waves lasting for 1/2 sec or more, consistently developped on the cortex and sometimes in many subcortical structures, it was practically absent in the mesencephalic reticular formation. Simultaneous bipolar recordings were made from the cortex and different subcortical sites, in order to compare for each location the relative amplitude of the recruiting waves and spindling after-activity. The results of such an experiment are illustrated in Fig. 14. Clear-cut recruitment may be seen in the cortical and reticular leads. Recruiting potentials built up as the number of repetitive shocks increased (Fig. 14 *B*) and the gains were adjusted in such a way as to give comparable amplitudes for the cortical and mesencephalic responses. Under these conditions, the absence of tripped spindle bursts in the reticular bipolar recordings was clearly evident. This absence was even noticed in some instances where spindling activity existed in other homolateral and heterolateral subcortical structures (record at the right in *A*).

We shall just mention here briefly the results of the unit analysis of reticular activity during the spindle patterns, because the main data have been already presented in details elsewhere (19, 21). The firing of cortical and thalamic neurons is often dependent on the smooth synchronized baseline activity so that the spontaneous spikes are grouped in high frequency bursts in phase-relation to the spindle waves (26). Such grouping has never been observed for mesencephalic nerve-cells in the course of systematic investigations. In conclusion, the modulation process described in Section 6, which appeared to be the more usual unit " response " of reticular neurons, does not exist under conditions of spontaneous synchronization. So far, we do not have any information concerning the other possibilities mentioned in Section 6 (e. g., activation, inhibition of firing) but, if present, these alterations must be relatively small. That does not mean however that they would be devoid of any importance.

DISCUSSION

The identity of the reticular potentials analyzed in these studies with classical thalamic recruiting processes, as they are commonly observed for instance on the cerebral cortex, has been already discussed in this paper and in other reports (20, 21). We think it requires no further comment.

First, what is the pathway for the conduction of these potentials from the thalamic to the ponto-mesencephalic and bulbar regions? The conservation of the responses after surgical or functional exclusion of the neopallium indicated that a neocortical relay is not necessarely implicated. In addition, a low-frequency stimulation carried in other structures than the unspecific thalamic nuclei (for instance the fornix, caudate nucleus, lateral thalamus and internal capsule) did not cause any classical recruitment in the mesencephalon. This point has to be mentioned here, although we do not considerer an absence of effect as a decisive argument against the existence of a thalamo-reticular pathway passing or relaying at the sites of stimulation. Indeed, stimulating the thalamic efferents cannot be regarded as equivalent to stimulating the thalamic neurons themselves. But, on the other hand, a limited lesion of the nervous tissue between the thalamic site of stimulation and the mesencephalic site of recording precluded the reticular recruiting responses. On this ground, we suggest the hypothesis of a direct projection going through the upper part of the centre median, at least for its main component. These hypothetical connections however cannot be related, at the present time, to any anatomically or physiologically known pathway.

The unit recruiting responses described in this report were generally recorded from mesencephalic and bulbar neurons which could also respond with a long latency to several kinds of sensory stimuli. On the other hand, at the completion of several experiments made with bipolar recording electrodes in the brain stem, these electrodes were used to apply a 100-200/sec stimulation, and this stimulation, even at low voltage (1-2 V), regularly induced a cortical desynchronization. On the basis of these two controls, we may probably assume that at least some proportion of the nerve cells which were found to display recruiting responses belonged to the so-called reticular ascending activating system. If our conclusions are correct up to this point, they would imply that the information on the activity of rostral structures which are under the control of the reticular system is transmitted back to this system. As such, this statement is quite factual. It only means that the neurons which can prevent the development of thalamic 6-12/sec rhythms are themselves influenced by these rhythmical potentials when they occur.

However this last conclusion is entirely based on the observation of recruiting responses in the brainstem. In contradistinction, we pointed out that the spontaneous spindles and tripped spindling after-activities were rare and small in the bipolar recordings from the reticular formation. Indeed, on many occasions, we consistently found a striking difference in the amplitude of spontaneous and evoked 6-12/sec cyclical potentials, but this difference seems to be really difficult to appreciate. It is much dependent upon the technical procedures of recording. For instance, the conditions of derivation (monopolar or bipolar), the exact position of the electrodes in the reticular formation and the distance between their tips may alter the size relation between the reticular spindles and recruiting responses. Further experments are thus required in order to analyze the development of spindles in the mesencephalon, and probably such investigations would throw more light on the role of thalamo-reticular projections.

SUMMARY

Experiments were performed in order to analyze the properties of recruiting responses induced in the mesencephalic and bulbar reticular formation by a repetitive stimulation of the unspecific thalamic nuclei, in unanesthetized cats.

Classical requirements (pattern organization, latency, optimum frequency of stimulation, sites of stimulation) were fulfilled to permit the identification of the reticular responses as a typical recruitment phenomenon. A clear-cut distinction was made with other repetitive responses having a different significance. Evidence was afforded that the cerebral cortex is not necessarily implicated in the transmission of the recruiting impulses from the thalamus to the brain stem. On the contrary, selective coagulations inside the thalamus tended to demonstrate that the pathway of conduction is a direct one, passing at the level of the upper portion of the centre median.

Microelectrode recordings from mesencephalic and bulbar units showed very complex patterns of responses which were described in details. A study of the convergence of thalamic and somesthetic impulses on reticular neurons discloses some properties of such an interaction. Lastly, the occurrence of recruiting responses in the mesencephalic reticular formation was compared with the slightness of spontaneous spindle bursts in the mesencephalon, under the conditions of these experiments.

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INTRACELLULAR DISTRIBUTION OF CHOLINESTERASE IN THE ANTERIOR HORN CELLS OF RAT [1]

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INTRODUCTION

Homogenates of tissues and their fractions represent the largest source of material for enzymological work since the description, ten years ago, of the methods for centrifugal fractionation. The use of this technique has, however, been criticized (8, 26, 32, 33, 37, 38, 48) and the need for comparison with other techniques has been stressed (2, 48).

A modification of the cartesian diver technique recently developed (21) for the determination of cholinesterase (ChE) [2] activity in $\mu\mu$l of cytoplasm and nucleoplasm made it possible to attempt a more exact intracellular localization of the enzyme. As the large anterior horn cells of the spinal cord of the rat have been found (17, 23) to be a convenient material for a detailed study of the distribution of ChE in the neurone they have been used in the present investigation.

It has been considered of interest to attempt to clarify the following points: *a*) to demonstrate by means of a sensitive biochemical technique the presence of acetylcholinesterase (AChE) in the nerve cell and to study its distribution quantitatively in the different parts of the neurone; *b)* to compare the amount of AChE activity in the anterior horn cells with that of other types of cells previously determined (18, 20, 24).

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[1] A preliminary report of the results of this investigation has been published in (19) and (21).

[2] Abbreviations: ChE = all enzymes splitting choline esters, irrespective of their specificity for certain substrates. AChE = acetylcholinesterase, specific cholinesterase, true cholinesterase, erythrocytes cholinesterase (" e " type), acetocholinesterase, Cholinesterase I. Non spec. ChE = non specific cholinesterase, pseudocholinesterase, serum cholinesterase (" s " type), butyro- and propionocholinesterase, Cholinesterase II.

METHODS

For the detailed description of the technique and material employed the reader is referred to a previous article (21).

The results of the experiments shown in Table I have been obtained by using the diver micropipette technique modified by the author, which has a sensitivity of 10^{-6} μl CO_2/hour ($\pm 15\%$ accuracy) (21).

The experiments reported in Table II were performed with the cartesian diver technique as previously reported (18). This technique has a sensitivity of 10^{-4} μl CO_2/hour ($\pm 5\%$ accuracy).

In some experiments reported in Table I and II the presence of AChE has been demonstrated by the help of Mipafox (bisaminophosphoryl iodide) a selective inhibitor of the non spec. ChE, at a concentration of 1×10^{-6} M. The technical procedure for the introduction of the inhibitor in the diver has been described (21). The substrate employed in all experiments was acetylcholine.

The addition of the inhibitor at the concentrations used gave a complete inhibition of the non spec. ChE under the experimental conditions described (21, 23).

The histochemical tests were performed on single nuclei preparation according to a modification of the thiocholine method of Koelle (17, 34, 36, 55). The incubation time was 90, 180 and 270 minutes.

RESULTS

1. *Experiments with single cell bodies.* — Table I shows the ChE activity curves of four rat anterior horn cells: the total ChE activity per cell is 1.3, 1.0, 1.2 and 7.2×10^{-3} μl CO_2/hour respectively; (the activity of cell no. 1 and no. 4 were shown by means of Mipafox to be due to AChE). Three of these cells have about the same volume (1.3, 1.4 and 1.2 $\mu^3 \times 10^3$) and the fourth somewhat higher (1.8 $\mu^3 \times 10^3$). It seems however that the ChE activity is not correlated with either the diameter, the volume or the area of the cell, and that cell no. 4 having a smaller volume has 5.5 times the activity per unit volume of the biggest cell. The significance of such a variation in ChE activity in different cells belonging to the same group has been discussed previously (22, 23).

2. *Experiments with isolated nuclei.* — In a previous investigation (17) it was found that when single nuclei isolated from anterior

ABLE I. – *ChE activity of single cell bodies and other portions of anterior horn cells of rat. Surface and volume values were calculated as previously reported (21). The sign + preceeds a figure expressing AChE activity.*

Exp. no.	Cell part	Diam. (μ)	Length (μ)	Surf. (μ^2) ($\times 10^3$)	Vol. (μ^3) ($\times 10^3$)	Total ChE act. μl CO_2/h. ($\times 10^{-6}$)	ChE act/ μl CO_2/h $\times \mu^2$ ($\times 10^{-7}$)	ChE act/ μl CO_2/h $\times \mu^3$ ($\times 10^{-9}$)
1	Cell body . .	28 × 16 × 8	—	0.74	1.8	+1322	17.9	736
2		31 × 9 × 9	—	0.58	1.32	1000	17.2	757
3		26 × 15 × 7	—	0.61	1.43	+1210	19.9	848
4		21 × 14 × 8	—	0.56	1.23	+7200	128.0	5750
5	Axon	1.3	40	0.16	0.053	+ 11	0.675	208
6		1.1	45	0.15	0.042	31	1.99	730
7		1.2	60	0.28	0.067	24	0.855	354
8		1.2	30	0.11	0.034	+ 180	16.5	5300
9	Dendrite . . .	1.6	35	0.17	0.070	+ 24	1.41	341
10		1.7	40	0.21	0.090	11	0.517	122
11		1.7	25	0.13	0.056	126	9.69	2250
12		1.9	25	0.14	0.071	160	10.70	2250
13	Nucleus . . .	7 × 4 × 3	—	0.060	0.043	2.2	0.36	50
14		8 × 4 × 3	—	0.066	0.050	2.3	0.35	46
15		9 × 4 × 2	—	0.054	0.037	+ 2.6	0.48	70
16		11 × 5 × 3	—	0.094	0.086	+ 5.1	0.54	59
17		11 × 4 × 3	—	0.081	0.069	5.6	0.69	81
18		12 × 5 × 4	—	0.12	0.120	7.8	0.64	62
19		15 × 6 × 3	—	0.13	0.140	8.6	0.65	61
20	Nucleolus . .	3.8	—	0.045	0.029	0.032	0.0073	1.1
21		4.2	—	—	—	no meas. activity		
22		3.9	—	—	—	no meas. activity		
23	Cytoplasm . .	—	—	—	0.01	+ 4.8	—	480
24		—	—	—	0.01	6.9	—	690
25		—	—	—	0.05	13.0	—	260
26		—	—	—	0.06	18.5	—	310
27	Nucleoplasm .	—	—	—	0.01	0.34	—	34
28		—	—	—	0.01	+ 0.41	—	41
29		—	—	—	0.04	1.24	—	31
30		—	—	—	0.10	4.90	—	49
31		—	—	—	0.09	5.40	—	60

horn cells were incubated for a short time (15-30 min) using the histochemical medium of Koelle (35) no crystal formation was observed. However, if small parts of cytoplasmic material were sticking to the nucleus these could give rise to precipitation of copper thiocholine sulphate crystals indicating the presence of ChE activity.

In the present investigation these findings were reinvestigated by means of the cartesian diver technique. The ChE activity was determined both in nuclei in which an accurate microscopical inspection in phase contrast showed no detectable cytoplasmic material sticking to the nucleus (Fig. 2 *D*) and in nuclei in which one or a few very small cytoplasmic particles were visible around the nuclear membrane (Fig. 2 *B*). The latter type of nucleus was either isolated by micromanipulation or was found in the suspension. The ChE activity was determined in 12 nuclei (see Table 2), 5 of these were cytoplasm free (n in Table 2) and 7 contamined with cytoplasmic material (n + g in Table 2). Table 2 demonstrates that all the contaminated nuclei showed some degree of ChE activity, varying between 29×10^{-6} and 594×10^{-6} μl CO_2/hour, but all the cytoplasm free nuclei showed no measurable activity (*i. e.*, less than 10^{-4} μl CO_2/hour).

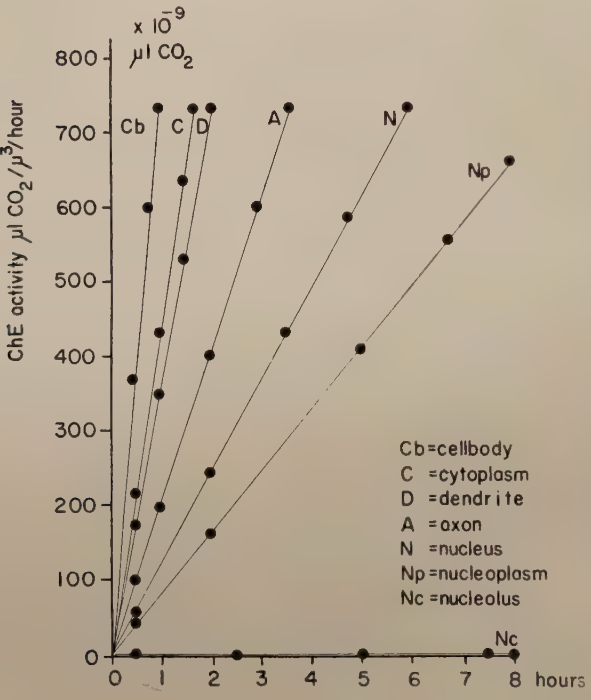

Fig. 1. – *ChE activity curves per unti volume (μ^3) of the cell body, cytoplasm, axon, dendrite, nucleus, nucleoplasm and nucleolus of a anterior horn cell of the spinal cord of the rat.*

The curves for the cell body, the axon and the dendrite refer to the same cell (see Table I, exp. 1, 5, 9); the curves for the nucleus, the cytoplasm and the nucleoplasm represents the mean ChE values calculated from Table I.

In histochemical experiments on single isolated nuclei lying in a drop of paraffin oil in which the incubation time was 2-3 hours, needle shaped crystals were visible all over the nuclear surface and inside the nucleus (Fig. 2 *C*) (21).

The ChE activity was therefore redetermined in 7 nuclei (Table 1) using a more sensitive diver technique (21). ChE was now detected in all nuclei, the activity varying between 2.2 and 8.6 × 10^{-6} μl CO_2/hour, corresponding to 28.6 and 111.8 pg ACh (1 pg = 10^{-12} g). The activity per unit volume of the nuclei varied between 0.04 and 0.08 × 10^{-6} μl CO_2/μ^3/hour. The ratio of activity per unit volume of the cell body and that of the nucleus varying approximately between 10 and 100. In calculating this ratio the possibility should however be taken into account that some of the nuclei examined belong to a group of anterior horn cells previously described (23) having a relatively high activity, and that for this reason the actual

TABLE II. – *ChE activity of single anterior horn cells* (c), *cytoplasm-free nuclei* (n), *nuclei contaminated with cytoplasm* (n + g) *and cytoplasmic granules* (g). *The ChE activity was calculated according to the formula previously reported* (21). *The sign + preceeds a figure expressing AChE activity.*

Exp. no.	Material	Total ChE activity μl CO_2/hour (× 10^{-6})
1	n + g	51
6	n + g	113
11	n + g	+ 594
12	n + g	+ 29
13	n + g	475
31	n + g	51
32	n + g	36
15	n	0
21	n	0
22	n	0
26	n	0
28	n	0
2	g	60
4	g	45
5	g	+ 78
7	g	186
8	g	+ 200
9	g	96
10	g	79
14	g	1000
16	g	1200
18	g	1545
19	c	+ 1680
23	c	1560
29	c	2400

range of variation of this ratio may be lower. As can be seen in Table 1 the activity per unit volume does not vary much from nucleus to nucleus and the activity cannot be directly correlated to the volume of the nucleus.

Some attempts were also made to determine the relationship between the ChE activity of the cytoplasm and that of the nucleus in the same cell, but owing to technical difficulties this proved impossible. The low ChE activity demonstrated in isolated nuclei both with the histochemical reaction after prolonged incubation and the cartesian diver technique cannot be taken as definitive proof of the presence of the enzyme in the nucleus, for there is the possibility that some residual contamination with cytoplasmic material

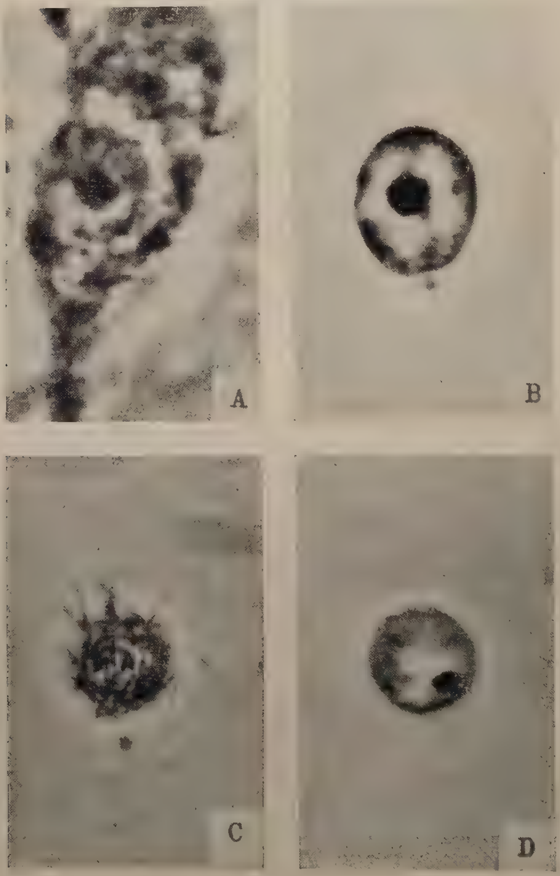

Fig. 2. – *A*: an anterior cell of the spinal cord of rat isolated by means of microdissection. *B*, *C*, *D*: single nucleus preparations isolated by anterior horn cells of rat. In *C* the ChE activity is demonstrated by thin needle shaped Cu-thiocholine crystals produced by the histochemical reaction and visible all over the nucleus. Incubation time 180 min. Magnification in *A*, *B*, *C*, *D* about 800 x.

occurs during the isolation procedure. In fact as shown in Table 2 both from the experiments with contaminated nuclei (n + g) and with isolated cytoplasmic granules (g), a very little amount of cytoplasm would be sufficient to show an activity of the order of that found in a single nucleus. It was therefore necessary to measure the ChE activity in samples of cytoplasm and nucleus directly removed from the isolated cell or nucleus by means of the micropipette technique (21). The result of these experiments are described in the next section.

3. *Experiments with nucleoplasmic material.* — Table 1 reports the results of 5 experiments done on samples of nucleoplasm removed from isolated nuclei preparations. The volume of the samples was estimated to lie between 0.1 and 0.01 $\mu\mu$l (1 $\mu\mu$l = 10^{-6} μl). All the nuclei investigated showed some ChE activity, varying between 0.3 and 5 × 10^{-6} μl CO_2/hour; the activity per unit volume varied between 31 × 10^{-9} μl CO_2/μ^3/hour and 60 × 10^{-9} μl CO_2/μ^3/hour and was of the same order of magnitude as that found in whole nucleus determinations (see table 1). The ChE activity of a 0.01 $\mu\mu$l sample of nucleoplasmic material, was previously found to be about 0.41 × 10^{-6} μl CO_2/hour corresponding to the hydrolysis of 5.3 pg ACh (21).

4. *Experiments with cytoplasmic material.* — During the first part of this investigation the ChE activity was determined in isolated nuclei as well as in cytoplasmic granules present in the cell suspension (the mean diameter of these granules varied between 1.6 and 2 μ) and also in cytoplasmic material isolated by means of micromanipulation from single cells. Under the phase contrast microscope the entire cytoplasm appeared to be filled with granules of various dimensions. These cytoplasmic granules could be stained with basic dyes (methylene blue 1: 10.000, toluidine blue and thionin) and were also present in chromatolitic cells.

Table 2 shows that the activity of these cytoplasmic granules (g) varies between 45 and 1545 × 10^{-6} μl CO_2/hour. These values have only semi-quantitative significance as it has not been possible to estimate quantitatively the amount of material investigated. For comparison the same table includes the ChE activity per cell of three cell bodies isolated from the same region (Fig. 2 *A*).

In Table 1 are reported the results of four experiments in which the ChE activity has been determined in small samples of cytoplasm removed from isolated cell bodies. It will be seen that the ChE activity per unit volume is in the same order as that calculated from the whole cell body experiments (see the same Table: cell body).

In a previous investigation (21) the ChE activity curve of a sample of cytoplasmic material of about 0.01 $\mu\mu l$ was shown to be 4.8×10^{-6} μl CO_2/hour, corresponding to the hydrolysis of 62.5 pg ACh.

5. *Experiments with isolated nucleoli.* — In three experiments the nucleoli were successfully isolated and introduced in the diver for the determination of ChE activity. Table 1 shows that only one of them had a measurable ChE activity of about 0.032×10^{-6} μl CO_2/hour, the mean diameter of this nucleolus was 3.8 u and the volume 0.029 $\mu^3 \times 10^3$. The possibility for a contamination with nuclear material and the sensitivity of the method employed should however be kept in mind when interpreting the results of these experiments.

6. *Experiments with isolated segments of axons and dendrites.* — In two experiments (see Table 1) the activity of the cytoplasm and the axoplasm was compared in the same cell. In the first cell (exp. no. 1-5, Table 1) the cell body showed an activity per unit volume of about three times greater than in the axon, and in the second cell (exp. no. 4-8, Table 1) the activity was about equal in the two parts. In two other experiments the activity per unit volume of the axon was the same or less than in the cell body.

The ChE activity was then determined in four segments of dendrites isolated from single cell preparations and was found approximately of the same order as that of the cell body. However, it should be pointed out that in one of these experiments the ChE activity was about 10 times higher than in the other; this suggests that the first group of dendrites was separated from cell bodies belonging to the group of cells having higher activity (23).

The values reported in Table 1 exp. 9 are those of a determination made on a segment of dendrite belonging to the same cell as exp. 1 and exp. 5. From the comparison of these results it becomes evident that a dendrite of this cell shows approximately half the ChE activity of the cell body but higher activity than its axon.

DISCUSSION

The results of this investigation together with those obtained by previous histochemical and biochemical work (17, 18, 19, 20, 21, 22, 23) demonstrate (with the help of a selective inhibitor) that AChE is present in some cells of sympathetic and spinal ganglia, and in all the anterior horn cells examined of the spinal cord of the rat. Non spec. ChE is not found in these cells with the exception of a low amount in the spinal and sympathetic ganglion cells (20, 18).

From the comparison of the data obtained by biochemical analysis of gross specimens of tissue (whole ganglions, anterior and posterior roots etc.) with the data obtained from quantitative determinations in single cells and cell parts in appears that AChE is concentrated in the neurones and in their processes.

Comparing the ChE activity per unit volume of cells of different types it can be seen that the activity of the anterior horn cells is 30-70 times higher than in the spinal ganglion cells (20) and 10-20 times higher than in the sympathetic ganglion cells (18).

In Fig. 1 is shown the AChE activity in a cell from the anterior horn of rat spinal cord expressed in terms of volume of the cell body, cytoplasm, axon, dendrite, nucleus, nucleoplasm and nucleolus.

From the results reported in Table 1 and Fig. 1 it is evident that the enzyme is not strictly localized in any one part of the anterior horn cell, but is present in varying amounts in different cell parts (Fig. 1 and Table 1). The cell body exhibits the highest values of ChE activity, the axon and the dendrites a somewhat lower one, while the nucleus has an activity 10-100 times lower than the other parts. The nucleolus showed in one experiment a very low activity.

The presence of the enzyme in the cytoplasm was demonstrated by means of two independent types of experiments and by determinations on samples removed from isolated cells.

The presence of the enzyme in the nucleus has been demonstrated by means of three independent techniques; first by a specific histochemical reaction on isolated nuclei, second by cartesian diver determinations in cytoplasm-free nuclei and cytoplasm contamined nuclei and finally in samples of nucleoplasm removed from isolated nuclei.

It was found that the ChE activity of the nucleus varied very little (between 50 and 81 × 10^{-9} μl CO_2/μ^3/hour) in contrast with that of the cytoplasm (between 736 and 5750 × 10^{-9} μl CO_2/μ^3/hour).

The recently advanced concept concerning the presence of holes 200-300 Å or more in diameter in the nuclear membrane of nerve cells (13, 45) and other cells (6, 7, 11, 30, 53) suggests that AChE molecules may pass from the nucleus to the cytoplasm under physiological conditions. Less probable is a process of simple diffusion of the enzyme in analogy with the findings of Dounce (15) and Anderson (3, 4) on isolated cell nuclei.

The possibility of the passage of the enzyme in the opposite direction, *i. e.*, from the cytoplasm to the nucleus, due to alteration of the nuclear membrane during the isolation procedure, has also to be taken into account as a possible source of artifacts. It has in fact been shown by Stern and Mirsky (50) by direct tests on sucrose prepared nuclei that an enzyme molecule can easily pass through the nuclear membrane probably due to changes occuring during the isolation procedure. The possible diffusion of non spec. ChE from the cytoplasm to the nuclei after sectioning has also been pointed out (35). Other tests have demonstrated that soluble enzymes can be extracted from nuclei by sucrose solution. The dimension of the cholinesterase molecule, its stability (25, 47), and other factors which have already been discussed (21, 22) together with the isolation procedure employed in this investigation (21) make it unlikely that the above objections apply in this case.

According to Dounce (14) the ChE in nuclei of liver cells should be about 50% of the concentration in the cytoplasm. Koelle (35) found that nuclei isolated from rat liver by the method of Dounce contained one quarter of the non spec. ChE. Goutier and Goutier Pirotte (27, 28) found in liver nuclei from albino rats that 22% of the non spec. ChE is present in the nuclei while only 7% of the AChE is present; most activity of both enzymes was found in the microsome fraction confirming the results of an earlier investigation of Glick *et al.* (44). Underhay, Holt, Beaufay and Duve (52), found the highest activity for cholinesterase in the microsome fraction of rat liver cells, the nuclei showed a moderate degree of activity and the mitocondrial fraction contained a very low activity.

The studies on enzymes from different fractions of nerve tissue are less numerous than those regarding liver cells. Isolated nuclei from the cerebral cortex obtained by means of grinding, straining and centrifuging have been employed for determination of ChE activity by Richter and Hullin (47). AChE was found in considerable concentration in the nuclear fraction, in eleven of the fourteen brains examined, the activity was greater in the nucleus than in the cytoplasm. It should however be considered that the nuclear fraction represents only 5,5% in weight of the tissue. Furthermore the nuclear fraction is the most heterogeneous, and besides nuclei it contains cytoplasmic particles, unbroken cells, erythrocytes, cell membranes, microsome and mitochondrial material. In addition alterations of the nuclear structure during the separation procedure has been demonstrated by electron microscopy (for references cf. 43). Regarding the nucleolus, no enzyme except the doubtful presence of phosphatases is presently known (9, 12, 54); in the present investigation in one case (three experiments) an isolated nucleolus showed very low ChE activity.

Nathan and Aprison (42) have recently investigated the ChE activity of the cytoplasmic particles isolated from brains of rabbits homogenized in sucrose and separated by means of centrifugal fractionation. They found that the fraction containing the large cytoplasmic particles and the mitochondria accounted for 70% of the total ChE activity, the microsome fraction contained 18% and had the highest N content. The soluble tissue fraction had only a negligible activity. Toschi (51) and Holmstedt and Toschi (31) could confirm the results of Nathan and Aprison by using homogenates of rat brain and finding the largest part of the ChE activity in the particulate cytoplasmic fractions (mitochondria and microsomes) and only a small amount in the nuclear fraction. The highest specific activity was found by Toschi (51) in the microsomal fraction. According to Toschi, a firm association exists between AChE and the 40-60 Å thick membraneous structures of this fraction. These membranes, according to electron microscopy (29) are considered to originate from the endoplasmic reticulum of neurones. Frontali and Toschi (16) found a similar association of ChE with membraneous structures in subcellular fractions from electric tissue from Torpedo. Smallman and Wolfe (49) found that in homogenates of insect nervous tissue most of the ChE is contained in the larger cell fragments although a part is localized in the microsome fraction. Aldridge and Johnson (1) investigated the ChE activity of different fractions of rat brain and found that upon high speed centrifuging 35-40% of the ChE sedimented in the microsomal fraction. There was however 27% of the ChE activity in the heavy mitochondrial fraction and 14% in the light mitochondria. The specific activity of non spec. ChE was found to be the highest in the nuclear fraction, though it was present in other fractions. These authors, discussing their results and comparing them with those obtained with the same technique in liver, conclude that considering that brain contains a great variety of cells of different types from large neurones down to microglia it is not surprising that less clear cut conclusions can be reached and point out that the present homogenization and centrifuging procedures may not be suited to separation of brain cell components.

Further support to a localization of the AChE in the cytoplasm comes from the studies of Brauer and Root (10), Paleus (46) and Augustinsson (5) which demonstrated that this enzyme is attached to the cell stroma of the erythrocytes and remains bound to it even after lysis of the erythrocytes with water.

The good correlation found with the diver experiments between ChE activity values of cytoplasm, whole cell bodies and nuclei is further evidence of the reliability of the data shown in the Table 1.

The results of a previous investigation (23) distinguishing between two groups of anterior horn cells depending upon their different ChE activity could be confirmed by the present investigation (see Table 1). The distribution pattern found in the cell body and in the axon of the anterior horn cells differs from that found in the spinal ganglion cells (20): in the former the concentration of the enzyme is the same or lower in the axon while in the latter the axon shows an activity 10-100 times higher. A migration process of the enzyme from the body to the axon could be responsible for the difference. It should also be emphasized that the axon of the ChE bearing cells of the spinal ganglions shows an activity of the same magnitude as that shown by the anterior horn cells (20).

The present investigation demonstrating the presence of AChE in the cytoplasm and in the nucleus of the nerve cell and the non correlation of the enzyme activity with the volume, the surface of the cell or of the axon does not support the previous view on the specific localization of the enzyme at the cell and axon surface (39, 40, 41).

SUMMARY

1. The ChE activity has been determined in the cell body, cytoplasm, axon, dendrite, nucleus, nucleoplasm and nucleolus of anterior horn cells of the spinal cord of rat by means of a sensitive cartesian diver technique.

2. The results demonstrate that ChE is highly concentrated in the neurones and their processes and that the enzyme is not strictly localized to a part of the cell but is present in different concentrations in the different parts.

3. The presence of the enzyme in the cytoplasm and in the nucleus was demonstrated by means of independent experiments and by two different techniques.

4. The results on the intracellular distribution of ChE in the neurone are discussed and compared with those of previous investigations on the distribution of enzymes in cellular particles obtained by means of fractionation methods.

ACKNOWLEDGMENTS

This investigation was supported by grants from the I. N. R. (Istituto Nazionale delle Ricerche), Rome, Italy and from the Karolinska Institutet, University of Stockholm, Stockholm, Sweden. An abstract of this paper was read before the Symposium on the histochemistry of the nervous system, New York, April 9, 1960.

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Arch. ital. Biol., 99: 163-177, 1961.

GALVANIC SKIN REACTIONS OF STRIATAL CATS WITH OR WITHOUT CEREBELLUM: EFFECTS THEREON OF ANESTHESIA [1]

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INTRODUCTION

During the half century from 1909 to 1959 all but three pieces of investigations on the galvanic skin reflex of animals (excluding man) were performed under anesthesia insofar as we know. The three exceptions were Veraguth's observations on the galvanic skin reflex in normal cats (held by a laboratory assistant) (28), Hara's tests of this reflex in cats and dogs under curare (15), and Richter's experiments on the reflex in spinal cats (25). Recently it has been pointed out by several authors (29, 5) that it is necessary to check the results of the experiments with anesthetized animals by doing the same experiments with unanesthetized animals. In view of the advances in the physiology of the central nervous system during the past 25 years, it is especially important to do so in the study of the central mechanisms of the galvanic skin reflex.

However, this is more easily said than done. Whether or not curarization has any effects on the central nervous system in animals (excluding man) is not definitely known (14). Hara (15) found it necessary to evoke the galvanic skin reflex in his curarized animals by electric stimulation of the proximal end of a severed sciatic nerve. The question as to why it is necessary to stimulate a sensory or mixed nerve instead of the sense organs in the skin in order to evoke

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[1] Supported by a grant (B-1460) from the National Institute of Neurological Diseases and Blindness, National Institutes of Health, Bethesda, Md., U.S.A.

the galvanic skin reflex in a curarized animal still awaits an answer. Veraguth's method of observation on the reflex in unanesthetized animals (28) is obviously not satisfactory, and has not been used by later workers. In the past year we have made observations on the galvanic skin reflex in unanesthetized normal cats by placing them in a hammock, which restrains their muscular movements to some extent. These cats were selected for their tameness. When placed in the hammock, nearly every one of them had its ears flattened, its head bent ventrad, its trunk curved dorsad, its limbs flexed, and its tail held between its hindlimbs and close to its abdomen. In short, all these cats were in a state of fright of different degrees. These experiments were not satisfactory for the following reasons: *a*) they dealt with specially selected animals; *b*) fear and muscular movements seemed to diminish the amplitude of the galvanic skin reflex, but the extent and mechanisms of the decrease in the reflex amplitude could not be ascertained with these cats.

In our unpublished work on the behavior of striatal cats—that is, cats with the neocortex removed on both sides but with the corpora striata intact—we have found that a striatal cat is very inactive. When put in a hammock for the first time, a striatal cat mildly struggled against restraint during the first 15-20 minutes and then became quiet for a long time, the longest being eight hours. After being in the hammock for two or three times, it was quiet even during the first 15-20 minutes. A striatal cat, therefore, is an excellent preparation for the study of the galvanic skin reflex in an unanesthetized animal and the effects thereon of anesthesia. Comparison of the reflex in such an animal with that in cats with intact brain or with the forebrain removed affords an opportunity of determining the role of the striatum (the caudate nucleus and putamen) in the production of the galvanic skin reflex. We have also found that, in contrast to a thalamic cat (12), a striatal cat survives decerebellation almost indefinitely. The part that the cerebellum plays in the production of the galvanic skin reflex may then be ascertained with long-surviving striatal cats with or without the cerebellum.

METHODS

Our experiments were performed with five striatal, one decerebellate-striatal, and two striatal-decerebellate cats. They were, as mentioned above, prepared for study of their behavior. The details concerning them will be published elsewhere. Their individual surgical histories are summarized in Table I. In this table, the first operation means removal of the neocortex on the left and the second operation removal of the neocortex on the right side and there is no third operation for the five striatal cats; the first two operations have the same meaning and the third operation means decerebellation for the two striatal-decerebellate cats; the first operation means decerebellation and the second and third operations mean removal of the neocortex on the left and right side, respectively, for the one decerebellate-striatal cat. The survival times varied from 88 to 333 days in these eight operated cats, and they are qualified to be called chronic preparations.

TABLE I

Nº of cat	Date of operations			Date of killing	Survival time in days
	First	Second	Third		
A. Striatal cats					
59-79	21/II/59	3/III/59	—	13/VIII/59	163
59-82	23/II/59	11/III/59	—	14/VIII/59	156
59-143	24/III/59	30/IV/59	—	17/VIII/59	109
59-350	29/VIII/59	24/IX/59	—	15/III/60	173
60-13	11/I/60	6/II/60	—	4/V/60	88
B. Striatal-decerebellate cats					
59-22	20/I/59	14/II/59	23/VIII/59	17/III/60	238
59-73	18/II/59	2/III/59	25/IV/59	23/III/60	333
C. Decerebellate-striatal cat					
58-180	12/V/58	6/IX/58	28/II/59	18/IX/59	202

After removal of the neocortex on both sides, each of these eight operated cats was tested for its galvanic skin reactions at irregular intervals. (We have coined the term, galvanic skin reaction, to include in one word: *a*) the galvanic skin reflex to stimulation of a sense organ or a sensory nerve, *b*) the galvanic skin response to stimulation of autonomic nerve fibers or an autonomic center, and *c*) the spontaneous variations in skin potentials. In the present paper we are concerned with the first and third of these reactions). At such a test, the cat was placed in a hammock, its four paws were connected to four channels of an Offner model T electroencephalograph by means of zinc electrodes used with $ZnSO_4$-kaolin paste, and a pair of stimulating electrodes (made of silver wires kept 1 cm apart and in the form of hooks) was fastened to the shaved skin of the tail near the tip. The spontaneous potential waves in the four paws were first recorded and the galvanic skin reflex was then tested. This reflex was registered as changes in skin potentials, and not as changes of skin resistence, of the footpads, and evoked by stimulation of the tail with tetanic shocks from a Harvard inductorium at the rate of one stimulation per minute. Just before each of the eight operated animals was killed we experimented on the effects of anesthesia on the two galvanic skin reactions. The galvanic skin reflex was evoked by stimulation of the tail before anesthesia, and by stimulation of the central end of the severed left superficial radial nerve afterwards. Tetanic shocks of the same parameters were applied once per minute in both stimulations. The anesthetics used were chloralose and urethane, the same as used in our previous acute experiments; and the dosage chosen (45 mg/kg of chloralose and 0.9 g/kg of urethane) was also the same. An aqueous solution of the two anesthetics was administered to the cat by intraperitoneal injection. At the end of the experiment the cat was killed and perfused with 4% formaldehyde in 1.25% saline solution. The brain was immediately removed for later histological study.

RESULTS

1. *Spontaneous potential waves.* — As described in a previous paper (17), spontaneous oscillations in skin potential occur simultaneously in the four paws of an unanesthetized chronic striatal cat.

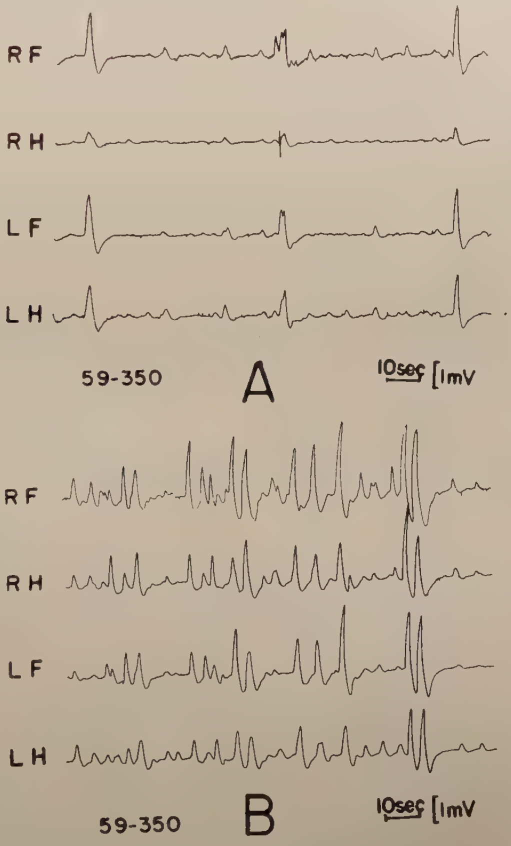

Fig. 1. - *Cat 59-350.* Record taken 125 days fater removal of neocortex on both sides. In this and following figure, RF: right forepaw; RH: right hindpaw; LF: left forepaw; LH: left hindpaw.

This phenomenon and its disappearence will be referred to in the present paper respectively as "synchronization" and "desynchronization". Examination of records of these waves taken at a speed of 1 mm/sec of the recording paper reveals the existence of two types of record. Fig. 1 *A* shows the first type, and Fig. 1 *B* and Fig. 2 *B* are examples of the second type. In the first type, most of the waves are low in amplitude with waves of high amplitude appearing at intervals; in the second type, nearly all waves are of high amplitude and the intervals between them are shorter than in the first type. The first type was usually obtained when the cat's eyes were closed; and the second type when the cat's eyes were open. The records of the spontaneous potential waves obtained from the striatal-decerebellate and decerebellate-striatal cats do not differ from the records obtained from striatal cats.

TABLE II

Nº of cat	Reflex[1] amplitude before anesth. in mV.	Reflex[2] amplitude during anesth. in mV.	Ratio Col. 2 to Col. 3	Abolition of reflex.[1] Min. after injection.	Desynchronization of waves. Min. after injection.
59-79	0.5	0.5	1.00 : 1.00	11	7-8
59-82	1.7	1.6	1.00 : 0.94	13	3-4
59-143	1.4	1.3	1.00 : 0.93	8	2-3
59-350	4.1	3.1	1.00 : 0.76	12	5-6
60-13	1.4	1.9	1.00 : 1.36	—	3-4
59-22	2.1	2.8	1.00 : 1.33	10	2-3
59-73	4.5	5.2	1.00 : 1.16	12	5-6
58-180	1.3	0.3	1.00 : 0.23	—	7-8

[1] galvanic skin reflex to stimulation of tail.
[2] galvanic skin reflex to stimulation of left superficial radial nerve.

2. *Galvanic skin reflex.* — Table 2 summarizes the findings on the eight operated cats on the day of sacrifice when each of them was tested for the two galvanic skin reactions both before and after administration of chloralose and urethane. Column 2 of this table gives for each cat the mean amplitude of the galvanic skin reflex from one forepaw to stimulation of the tail before administration of the two anesthetics. The mean amplitude was calculated from at least five observations. All but two of the eight cats have a reflex amplitude lower than 2.5 mV. The exceptions are cat 59-350, a striatal preparation and cat 59-73, a striatal-decerebellate. Their mean reflex amplitude is slightly higher than 4.0 mV. Unfortunately, there is no reliable information on the mean amplitude of the galvanic skin reflex in normal cats not under anesthesia. In a large number of normal cats under chloralose and urethane, we have found that

the mean amplitude of the reflex from forepaws varies from 0.5 to 30.0 mV (32, 29). In one thalamic cat that survived 333 days after removal of the forebrain on both sides, a single shock either delivered to the tail when it was not under chloralose and urethane, or applied to the bared left superficial radial nerve when it was under the two anesthetics, always elicited a galvanic skin reflex of an amplitude as high as did tetanic shocks (29). A single shock usually fails to elicit the galvanic skin reflex in striatal cats. We feel justified in stating that striatal, striatal-decerebellate, and decerebellate-striatal cats have low mean amplitude of the galvanic skin reflex.

3. *Effects of chloralose and urethane.* — a) *On spontaneous potential waves in four paws.* Fig. 2 presents three stretches of record of the spontaneous potential waves from the four paws of a striatal cat taken 88 days after the second operation. Fig. 2 *A* was taken 42 minutes before administration of chloralose and urethane, *B* 2-4 minutes after, and *C* 6-7 minutes after. This cat (60-13) has the second type of spontaneous potential waves, mentioned above, the waves from the four paws being synchronized and having high amplitude with short intervals between individual waves. Between the third and fourth minutes after administration of the two anesthetics, as indicated by the arrow in Fig. 2 *B*, the waves from the four paws are suddendly desynchronized and remain so to near the end of the experiment. Comparison of *A* and *C* in Fig. 2 brings out another effect of anesthesia on the spontaneous potential waves from the four paws, a large decrease in the amplitude of the waves. The waves from the four paws were progressively but not simultaneously reduced to zero near the end of experiment about 90 minutes after the start of anesthesia. Desynchronization of spontaneous potential waves from the four paws and progressive decrease of their amplitude were observed in all striatal, striatal-decerebellate, and decerebellate-striatal cats. Here it should be emphasized that the two changes in the spontaneous potential waves occurred soon after injection of chloralose and urethane, the earliest being between two and three minutes after injection while the latest happened between seven and eight minutes after (see column 6 in Table 2).

b) *On galvanic skin reflex.* Column 5 of Table 2 shows that the galvanic skin reflex to stimulation of the tail is abolished in six of the eight operated cats, in 8-13 minutes (mean = 11 minutes) after

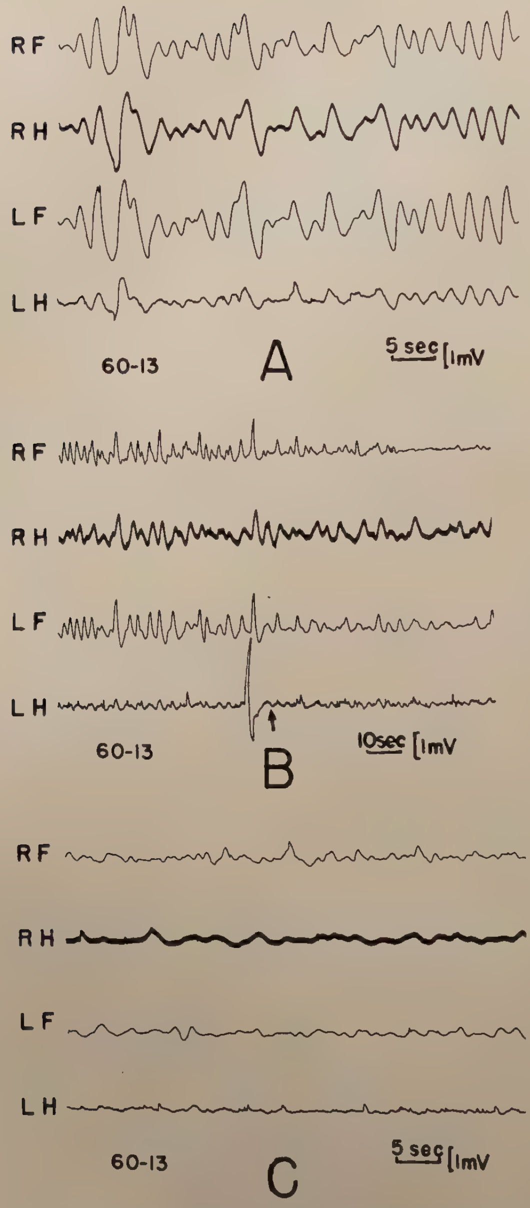

Fig. 2. - *Cat 60-13*. Record taken 88 days after removal of neocortex on both sides.

administration of chloralose and urethane, *i. e.* several minutes after abolition of the spontaneous synchronous oscellations of the skin potential (column 6). Indeed the reflex response to stimulation of the tail was not abolished in cat 60-13 even at the end of the experiment (about 90 minutes after injection of the two anesthetics). Cat 58-180 received in the course of experiment, three injections so that the time of abolition of the reflex to stimulation of the tail cannot be accurately determined. After abolition of the reflex to stimulation of tail, stimulation of the central end of the divided left superficial radial nerve still elicits the reflex. The reflex elicited by stimulation of the whole trunk of a cutaneous nerve, as the superficial radial nerve, should be expected to have a higher amplitude than the reflex elicited by stimulation of a small skin area on the tail. It is not so. In all but one of the eight operated cats, the amplitude of the two reflexes is approximately the same (see column 4 of Table 2, which gives the ratios between column 2 and column 3 of the same table). Cat 58-180 received 160 mg of chloralose and 3.2 g of urethane in three injections. With this cat, stimulation of the left superficial radial nerve during anesthesia evoked a galvanic skin reflex, whose mean amplitude corresponded 23 % of the mean amplitude of the same reflex evoked by stimulation of the tail before anesthesia. It might well have been due to the large dose of anesthetics cat 58-180 had received.

To summarize the effects of chloralose and urethane on the two galvanic skin reactions in striatal, striatal-decerebellate, and decerebellate-striatal cats: *a*) they desynchronize the spontaneous potential waves from the four paws and progressively decrease the amplitude of the waves to zero; *b*) they abolish the galvanic skin reflex to stimulation of the tail but not to stimulation of the central end of a divided cutaneous nerve; and they produce no large difference in amplitude of these two types of the reflex, unless their dosage is large; *c*) they first desynchronize the spontaneous potential waves from the four paws and then abolish the galvanic skin reflex to stimulation of the tail.

Discussion

1. *Cerebellum and galvanic skin reactions.* — Stimulation of the anterior lobe of the cerebellum inhibits vasomotor reflexes (21), the somatic and autonomic reactions during sham rage in acute thalamic cats (22), and the galvanic skin reflex (30). Thus far no one except Bard *et al.* (4) seems to have succeeded in demonstrating the effects, if any, of removal of the cerebellum on any particular autonomic reflex (7). We hoped that a striatal cat with the cerebellum removed

might show some differences in galvanic skin reactions from a striatal cat with the cerebellum intact, if both cats were kept alive for a sufficiently long time. The two striatal-decerebellate cats (59-22, and 59-73) survived the third operation for 238 and 333 days, respectively. The one decerebellate-striatal cat (58-180) survived the third operation for 202 days. All these three cats survived much longer than Rademaker's two decorticate-decerebellate dogs (' Robbie ' and ' Neger ') (24, 20, 26), which survived removal of both cerebral hemispheres and cerebellum for 38 and 89 days, respectively. In spite of their long survival, our three striatal cats without cerebellum did not differ from our five striatal cats with cerebellum in galvanic skin reactions. Once again, an attempt to show the effects of decerebellation on autonomic reactions has failed.

2. *Striatum and galvanic skin reactions.* — Stimulation of the striatum inhibits muscular movements induced by cortical stimulation (19, 9, 13). It also inhibits such autonomic reactions as contraction of the urinary bladder (9) and the galvanic skin reflex (9, 30). It is expected, then that a striatal cat should have a galvanic skin reflex of low amplitude. This is what we found and described in the preceding section.

3. *Anesthesia and galvanic skin reflex.* — In this part of discussion we are confronted with the following two questions: *a*) how it happens that anesthesia abolishes the galvanic skin reflex to stimulation of the nociceptors in the skin of the tail but not to stimulation of the central end of a severed cutaneous nerve; and *b*) how it happens that the galvanic skin reflex of our eight cats is of low amplitude both before and after administration of chloralose and urethane.

Either electric stimulation of the central end of a divided cutaneous nerve or tactile stimulation of the skin induces an evoked potential in the somesthetic area of the cerebral cortex in mammals under anesthesia (33, 34). Both stimulations induce discharges from pyramidal cells of the cerebral cortex to the spinal cord (3, 18, 23) in mammals under anesthesia. Yet stimulation of the receptors in the skin does not elicit any reflex either somatic or autonomic in anesthetized animals. The reason for this may probably be found in the following experimental facts. There are two sensory systems in the brain-namely, the classical lemniscal pathway and the pathway through the central reticular formation (10). Conduction in the latter is abolished by anesthesia (11). It seems necessary to have both pathways functioning in order to evoke a reflex either somatic or autonomic by sensory stimulation.

Even under anesthesia there is discharge of impulses in the pyramidal tract of mammals (1), and also in the reticular formation (2, 16, 27). It may be assumed that there are discharges of descending impulses from all structures at different levels of the brain to spinal motoneurons in anesthetized animals. Reflexes (the galvanic skin reflex included) elicited by stimulation of a sensory nerve in such animals are the results of algebraic summation of the descending impulses from the structures at different levels of the brain and of the incoming impulses from the periphery set up by stimulation of the sensory nerve, the central and peripheral impulses meeting in the pools of motoneurons in the spinal cord. Removal of the neocortex deprives a striatal cat of two excitatory centers (the sensorimotor area and infralimbic area of the cerebral cortex) and one inhibitory center (the frontal lobe of the cerebral cortex) for the galvanic skin reflex. It leaves intact two excitatory centers (the dorsomedial area of the dorsal thalamus and the anterior hypothalamus), and three inhibitory centers (the caudate nucleus, the cerebellar anterior lobe and the bulbar ventromedial reticular formation). Presumably the inhibitory influences predominate over the excitatory ones for the galvanic reflex in a striatal cat. The amplitude of the galvanic skin reflex to stimulation of a sensory nerve is a striatal cat consequently is low. An alternate view may be formulated on the peculiar action of chloralose, one of the two anesthetics we have used in our acute experiments since 1954. Chloralose is known to enhance the discharges from many structures in the brain (8). However this view does not seem likely. The results of old works upon the effects of transections of the central neuraxis on the galvanic skin reflex in cats under ether (6, 31) are not fundamentally different from the results of recent similar work with cats under chloralose and urethane (32).

4. *Structure in brain responsible for synchronization of spontaneous potential waves from four paws.* — In a previous paper (17) we have suggested that the structure in the brain responsible for synchronization of the spontaneous potential waves from the four paws in an unanesthetized cat lay in the interbrain. In the present paper we have described that chloralose and urethane desynchronize the waves from the four paws in from two to eight minutes after their administration in all eight cats. On the assumption that

anesthetization by these two anesthetics proceeds from the forebrain to the hindbrain, the quick desynchronization of the waves from the four paws after their administration indicates that the structure responsible for synchronization of the waves from the four paws is not far caudal to the striatum.

SUMMARY

1. Observations were made on two galvanic skin reactions of five striatal, one decerebellate-striatal, and two striatal-decerebellate cats, and the effects thereon of administration of chloralose and urethane.

2. When not under chloralose and urethane, these eight cats showed two types of synchronized spontaneous potential waves from the four paws. The first type, observed when the cat's eyes were closed, consisted of mostly waves of low amplitude with waves of high amplitude appearing at long intervals; and the second type, observed when the cat's eyes were open, comprised of waves of high amplitude appearing at short intervals.

3. When not under chloralose and urethane, the eight cats exhibited a galvanic skin reflex of low amplitude to stimulation of the tail.

4. Chloralose and urethane desynchronized the spontaneous potential waves from the four paws in two to eight minutes after their administration, and also gradually decreased the amplitude of the waves to zero.

5. Chloralose and urethane abolished the galvanic skin reflex to stimulation of the tail, but not to stimulation of the central end of a severed cutaneous nerve.

6. The galvanic skin reflex to stimulation of a sensory cutaneous nerve after administration of chloralose and urethane had the same low amplitude as the reflex to stimulation of the tail before anesthesia.

7. Chloralose and urethane desynchronized the spontaneous potential waves from the four paws first and abolished the galvanic skin reflex to stimulation of the tail later.

ACKNOWLEDGMENTS

I am indebted to Dr. Clinton N. Woolsey for support and encouragement; to Drs. Roongtam Ladpli, Kenan Umar, and Fabián Isamat de la Riva for assistance at different stages of work reported in the paper; and to Miss Helen Brandemuehl for making the photographs.

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Arch. ital. Biol., *99*: *178-190*, *1961.*

THE METABOLIC POSITION OF TRANSAMINASES IN THE NERVOUS SYSTEM

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INTRODUCTION

The purpose of the present report is to summarize some of the existing experimental data on transaminases in the nervous tissue and to discuss few hypothesis. In recent years, numerous investigations have been performed on transaminases of the cerebro-spinal fluid (see for references, 16, 80), but their results do not add to an understanding of biochemical mechanisms in the nervous tissue or in the cerebro-spinal fluid. Moreover, evidence of their clinical application is not available as yet.

The component reactions of the metabolic system involving transaminases are not fully known. This points out the need for studies on purified enzymes, though these studies would not directly reveal the real mechanism of metabolic regulation in neurons and glial cells. As noted by Krebs (37), one of the main problems in the analysis of metabolic regulation is the identification of those component reactions which are rate-limiting. This rate-limiting step is not known in the system involving transaminases. This paper will show that a control by feedback (37) is really operating.

General discussion of transamination reactions have been written and are therefore omitted here (see for references, 14, 18, 20, 54).

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I. – *Transaminases and the nervous system.*[1]

Table I summarizes general types of transamination reactions according to Fruton and Simmonds (29). Type I has been found to be widespread among several species, while the other types seem to be less ubiquitous. Type 3 *a* is of particular neurochemical significance since Bessman *et al.* (8) have shown it to occur mainly in brain. On the contrary, type 4 *a* has not been shown to be carried out by the nervous tissue, although the possibility of its participation to the brain metabolism has not been eliminated. In any case, if performed by the nervous tissue, it is catalyzed by an enzyme different from glutamic-oxaloacetic transaminase (GOT), recently pu-

TABLE I. – *General types of transamination reactions* (29).

Type	General Reactions
I	L-α-Amino acid + α-ketoglutaric acid $\rightleftarrows$ α-Keto acid + L-glutamic acid
2	D-α-Amino acid + α-ketoglutaric acid $\rightleftarrows$ α-Keto acid + D-glutamic acid
3	*a*) γ-Aminobutyric acid + α-ketoglutaric acid $\rightleftarrows$ Succinic semialdehyde + L-glutamic acid
	b) L-Ornithine + α-keto acid $\rightleftarrows$ L-Glutamic-γ-semialdehyde + L-α-amino acid
4	*a*) L-Glutamine + α-keto acid $\rightleftarrows$ α-Ketoglutaramic acid + L-α-amino acid
	b) L-Asparagine + α-keto acid $\rightleftarrows$ α-Ketosuccinamic acid + L-α-amino acid
5	L-α-Amino monocarboxylic acid$_1$ + α-keto monocarboxylic acid$_2$ $\rightleftarrows$ α-Keto monocarboxylic acid$_1$ + L-α-amino monocarboxylic acid$_2$

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[1] The abbreviations used are: ABAT: γ-aminobutyric-α-ketoglutaric acid transaminase; ATP: adenosine-5'-triphosphate; DPN: diphosphopyridine nucleotide; GDC: glutamic acid decarboxylase; GDH: glutamic acid dehydrogenase; GOT: glutamic oxaloacetic transaminase; GPT: glutamic pyruvic transaminase; LDH: lactic acid dehydrogenase; MDH: malic acid dehydrogenase; Py5P: pyridoxal 5-phosphate; Py5PINH: *iso*nicotinoylhydrazone of pyridoxal 5-phosphate.

rified from human brain (9). The possibility of a transamination involving the-α-amino group of glutamine needs to be further investigated in brain. Lestrovaia (42) has reported, in fact, that glutamine is formed in brain preparations after addition of glutamic acid and asparagine.

The following two reactions (type 1 and type 3 *a* in Table I) have been the most extensively studied in the field of brain transaminations:

$L(+)$glutamic acid + oxaloacetic acid $\rightleftarrows$ α-ketoglutaric acid + $L(+)$aspartic acid (reaction 1);

$L(+)$glutamic acid + succinic semialdehyde $\rightleftarrows$ α-ketoglutaric acid + γ-aminobutyric acid (reaction 2).

Reaction 3 (type 1 in Table I) has been the subject of only minor investigations with brain homogenates:

$L(+)$glutamic acid + pyruvic acid $\rightleftarrows$ α-ketoglutaric acid + $L(+)$alanine (reaction 3).

Despite species differences (76), the rate of reaction 3 is generally low in brain (21). Conversely, the rate of reaction 1 is very high, heart and skeletal muscle being the only organs which contain a higher amount of GOT. In view of its wide distribution and of its abundance in many tissues, Cohen (20) defined reaction 1 as a *key transamination system.* The following discussion demonstrates the validity and the limits of such a definition with reference to the nervous tissue.

Ornithine is not substrate of transamination in brain (59)[1]. Conversely, rat brain homogenates do catalyze the transamination between α-ketoglutaric acid and the following aminoacids: aspartic acid, alanine, leucine, *iso*-leucine and valine (3). The ratios for the production of glutamic acid/g fresh brain/hr were, in a typical experiment, the following: 264: 154: 34: 21: 11. As reported by McIlwain (52), β-alanine is another substrate of transamination in the nervous tissue. However, among so many reactions, transaminations 1 and 2 were the only ones investigated more carefully.

GOT has been recently purified both from pig heart (30, 32, 43) and human brain (9). The enzyme from the latter source shows some remarkable peculiarities, which will be described in the following pages. It seems that in human brain pyridoxal 5-phosphate (Py5P) in less firmly bound to the apo-transaminase than in pig heart.

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[1] Glutamic semialdehyde is formed in other tissues (59).

Although they are more drastic, the procedures of Jenkins and Sizer (30) and Jenkins *et al.* (32) for purifying GOT from pig heart achieve much less resolution than does the technique by Bonavita for brain GOT (9). One wonders whether the minor stability of the coenzyme binding is only apparent; it cannot be ruled out as yet the protection of pig heart GOT on part of other materials present in the homogenate submitted to heating. The highest specific activity obtained for GOT from human brain is 20.6 (μ-moles of oxaloacetic acid formed per 1 mg protein per min, at 37°C), and this value is definitely lower than that observed for the enzyme from pig heart (30,32). The discrepancy may be due to an insufficient purification of the brain GOT, but it is also possible that the brain GOT has a lower specific activity than the enzyme from pig heart. It is worth noting that the value reported above for the specific activity of GOT in human brain may well be lower than the true value, since GOT cannot be completely reactivated after resolution (17).

The pH optimum for human brain GOT activity was found to be at pH 7.1. In this respect, GOT from human brain differs from the enzyme of other tissues. Jenkins *et al.* (32) observed a plateau from pH 8 to 8.5 in the case of pig heart GOT. Roberts *et al.* (65) have observed a similar plateau between pH 8 and 8.4 with the enzyme from the giant axon of the squid *Loligo pealii.* Conversely, in the rabbit brain the pH optimum (7.7) is closer (65) to the value reported for human brain (9). pK'_a values for GOT from human brain ($pK'_a = 5.7$) and pig heart ($pK'_a = 6.3$) are also different.

Concerning the coenzyme function of phosphopyridoxamine, it will be recalled that Meister *et al.* (55) and Jenkins *et al.* (32) were able to activate the apo-transaminase from pig heart through prolonged incubation with phosphopyridoxamine. This was not observed in the case of the brain enzyme (9). The easiest explanation is to assume that different alterations take place on the apo-protein molecule according to the purification procedures applied, while the presence in pyridoxamine 5-phosphate of an amino group with $pK'_a = 10.92$ (79) does not facilitate the interaction of the compound with protein molecules. In any case, further investigation is required on this point before the observed difference is regarded as tissue specific.

Similarly, the existing data on the thermodynamics of reaction 1 catalyzed by GOT from human brain do not agree with results obtained by using pig heart GOT. In the case of the brain enzyme, the activation energy for the transamination between *L*(+)aspartic acid and α-ketoglutaric acid was found to be 5,960 cal/mole over the temperature range between 25° and 40°C (9), while Nisonoff and Barnes (57) determined a value of 12,500 cal-mole for hog heart GOT. Experimental conditions, however, were not identical.

With human brain GOT, K_m values for *L*(+)glutamic acid and *L*(+)aspartic acid were found to be equal to 1.82 and 1.67 × × 10^{-3} M (9), while with pig heart GOT Jenkins *et al.* (32) determined a K_m value of 3.9 × 10^{-3} M, which is in good agreement with the value calculated on the data by Nisonoff and Barnes relative to the hog heart enzyme (57). Roberts *et al.* (65) reported K_m values of 2.6 × 10^{-3} M for *L*(+)aspartic acid with the rabbit brain enzyme and 0.5 × 10^{-3} M for the enzyme in the giant squid axoplasm. It is worth noting that with both the last two enzymes the K_m for α-ketoglutaric acid is much lower than the K_m for aspartate (0.59 and 0.2 × 10^{-3} M, respectively).

No further differences between the brain enzyme and the enzyme from other sources were found. It should be stressed, however, that the lack of investigations on GOT from other human or rabbit tissues does not permit very definite conclusions regarding the significance of the findings detailed above in terms of species differences versus organ differences.

Reaction 2 was first described by Bessman *et al.* (8) in brain homogenates from rat, rabbit and beef. The rate of reaction 2 is definitely lower in the liver, while kidney does not seem to contain the enzyme. Bessman *et al.* (8) were unable to show a stochiometric relationship between the production of *L*(+)glutamic acid and succinic semialdehyde, and they assumed that the latter compound was anaerobically destroyed or spontaneously polymerized. These doubts have been overcome, however, by the subsequent investigation of Baxter and Roberts (5). The enzyme which catalyzes reaction 2 is more abundant in the grey matter of beef brain than in the white matter. Mice brains contain still more enzyme. The pH optimum is near 8.2 and K_m values for γ-aminobutyric acid and α-ketoglutaric acid are 3 × 10^{-3} and 4 × 10^{-3} M, respectively. Phosphopyridoxamine was not tested as a coenzyme, but pyridoxal 5-phosphate was able to activate the enzymic protein from 20 to 130% depending upon the procedure used for purifying the enzyme. Unfortunately, no data are available on the same enzyme from other sources [1]. However, in the enzyme from acetate adapted *Escherichia coli* the binding between the apo-protein and phosphopyridoxal is definitely firmer than in the brain enzyme. This results parallels the observation reported above (9) concerning the GOT from human brain and from pig heart. The topics of the differential affinity of pyridoxal 5-phosphate for apo-enzymes appears to be physiologically important, since it is concerned with the competition of proteins for the coenzyme. As is known, pyridoxal 5-phosphate can react also with serum albumin (19): thus, in the absence of a higher affinity for enzymic proteins the economy of vitamin B_6 would be seriously affected.

Quantitative histochemistry has given few but remarkable contributions to the field of brain transaminases. By these methods GOT has been found to be more abundant in neurons than in capsules of gangliar cells and myelinic fibers. Furthermore, Lowry *et al.* (46, 47, 48) have shown a close association of GOT and malic dehydrogenases (MDH) in these tissues as well as in the retina of monkeys and white rabbits [2]. The ratio of MDH to GOT appears to remain constant in all areas of the monkey brain. It has been suggested (47) that small amounts of malic acid could trigger the conversion of glutamic to aspartic acid plus CO_2, according to scheme 1.

SCHEME 1 (47)

$$\text{Malate} \xrightarrow{\text{MDH}} \text{oxaloacetate}$$

$$\text{Oxaloacetate} + \text{glutamate} \xrightarrow{\text{GOT}} \text{aspartate} + \alpha\text{-ketoglutarate}$$

$$\alpha\text{-Ketoglutarate} \dashrightarrow \text{malate} + CO_2$$

This would be regarded as an emergency mechanism for the use of glutamate when other substrates are lacking. Another finding of the histochemical studies on retina by Lowry *et al.* (47) is the segregation of MDH from lactic dehydrogenase (LDH) in several retinal layers. In general, when MDH is high, LDH is low. If LDH is considered as an index of glycolytic capacity, one would think that an oxidative metabolism prevails where LDH is low and MDH and GOT are high.

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[1] Scott and Jacoby (67) have purified the enzyme from *Pseudomonas*, but they have not carried out a complete study of its properties.

[2] The lack of informations concerning the behaviour of GOT during the development of the nervous system can be tentatively balanced by considering data on MDH. Kuhlman and Lowry (40) observed a three fold increase in MDH and GDH activity from birth to maturity of rat brain. In view of the metabolic relation between GOT and MDH one wonders whether GOT exhibits a similar time curve during brain development. Concerning GPT, it is known that it achieves the highest value in rabbit brain between the 17th and the 29th day after birth (68).

Lowry (46) has investigated also the distribution of GOT in different structures of the nervous system. The highest values have been found in the dorsal root ganglion cells of rabbit (31.3 ± 2.2 moles of substrate converted per kilo dry weight per hour), while the lowest values in the capsules of dorsal root ganglion cells (4.9 ± 0.6) and in the white fibers within the dorsal root ganglions (2.4 ± 0.2). High values of GOT have been found by McDougal (51) in the optic tract and in the mammillo-thalamic tract, while the dorsal columns and the olfactory tract were definitely lower.

The association of MDH and GOT has been observed also in the giant squid axon, constituting a further evidence of a subcycle in the nervous tissue as proposed by Roberts *et al.* (65). Finally, it is worth noting that the distribution of fumarase also parallels the GOT distribution (51).

Quantitative histochemical data are available now on the distribution of γ-aminobutyric acid-α-ketoglutaric acid transaminase (ABAT) as investigated by Salvador and Albers (66). While the values reported for white tracts and peripheral nerves are " so low to be of doubtful significance ", the grey matter is rich in enzyme, with striking differences also in areas structurally similar. The most interesting finding of Salvador and Albers (66) seems to be the presence in the central nervous system of a rostral-caudal gradient with the highest values of ABAT activity in the brain stem and spinal cord. Only few structures like the red nucleus and the *substantia nigra* do not fit in the gradient and exhinit a reaction rate which is lower than in the surrounding parts. Albers and Brady (2) were not able to find the same gradient[1] for glutamic decarboxylase (GDC), which showed rather distinctively high values in cerebral cortex, cerebellar cortex and the *substantia nigra.* These same structures exhibit moderate or low ABAT activity. As calculated by Albers and Brady (2), the ratio of ABAT to GDC is greater than 1 in all areas, but marked differences are found (the ratio is equal to 52 in the inferior olivary nucleus and 1.4 in the zona reticularis of the *substantia nigra*). One would think that the limiting reaction as shown in scheme 2 is the decarboxylation of glutamic acid [1].

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[1] A ventral-dorsal gradient has been observed for ABAT and GDC (66, 2).

SCHEME 2 (66)

$$\text{Glutamate} \longrightarrow \gamma\text{-aminobutyrate} + CO_2$$

$$\gamma\text{-Aminobutyrate} + \alpha\text{-ketoglutarate} \longrightarrow \text{succinic semialdehyde} + \text{glutamate}$$

$$\text{Succinic semialdehyde} + \text{DPN} \longrightarrow \text{succinate} + \text{DPNH}$$

The histochemical findings detailed above prompt some critical considerations. It has been postulated that the relative amount of an enzyme in a particular structure is " an index of the prominence of that metabolic step and of the metabolic pathway to which that enzyme belongs" (46). However, the content of a definite enzyme in a neural structure can be high on a weight basis, and the actual rate of the reaction catalyzed can still be low, owing to a low affinity of the enzyme for its natural substrate(s), and/or to a rate-limiting step, and/or to the presence of a natural inhibitor. These are only few of the factors which determine the actual rate of an enzymatic reaction *in vivo* as well as *in vitro*.

As a last point in the discussion of brain transaminases, one more biochemical peculiarity of the nervous tissue will be recalled, *i.e.* the acetylation of aspartic acid as described by Tallan *et al.* (73). This reaction seems to be carried out only by the nervous tissue, but its metabolic significance is still uncertain. It is remarkable, however, that a high percentage of brain aspartic acid is present in the acetylated form (72).

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[1] Since GDC is the enzyme generating γ-aminobutyric acid (factor I), it was supposed (44) that its distribution would parallel the distribution of inhibitory synapses. For instance, cerebellar cortex should contain less GDC, since it is thought to have less inhibitory synapses (60). Lowe *et al.* (44) did find such a difference, but certainly its magnitude is not striking. As a matter of fact, the metabolic position of GDC requires fuller investigation.

II. – Metabolic pathways

Several properties of GOT and ABAT in the nervous system have been described in the first section of this paper. The following discussion is aimed at emphasizing the crucial position of these two enzymes and giving an integrated metabolic pattern in the nervous system. As a matter of fact, the leading role of GOT and ABAT can be easily inferred from the extraordinary abundance in the brain of glutamic and γ-aminobutyric acids. Tower (74) has drawn some tentative conclusions on the distribution of glutamic acid, glutamine, and γ-aminobutyric acid in different brain structures. Accordingly, all the cortical glutamic and γ-aminobutyric acid seem to be associated with neurons and primarily with mitochondria, as shown by differential centrifugation, while glutamine does not prevail in mitochondria and half of it in the cortical areas appears to be associated with non-neuronal elements. Actually, the localization of glutamic and γ-aminobutyric acids parallels the cellular distribution of glutamic dehygrogenase (GDH). Conversely, GOT is a soluble enzyme, although it is functionally related to the Krebs cycle, whose enzymes are associated with mitochondria.

Since the abundance of glutamic acid in the nervous system is unique [1], many of the major peculiarities concerning the transaminases can be inferred from a close analysis of the glutamic acid metabolism in the brain. Scheme 3 represents the metabolic position of $L(+)$glutamic acid in the nervous tissue and shows how the transamination between glutamic and oxaloacetic acid is one among six enzymatic tramsformations which the conpound can undergo. Since reactions shown in Scheme 3 are reversible, glutamic acid can be considered as a point of departure as well as of arrival. In view of the high concentration of γ-aminobutyric acid in the nervous tissue, one would suppose that the decarboxylation of glutamic acid is more active than elsewhere. The experimental evidence is consistent with this hypothesis (76). Scheme 3 does not represent any transamination reaction for glutamine; Meister and Tice (56) did not show production in brain of α-ketoglutaramic acid. On the contrary, even if glutamine is not a substrate of transamination in the nervous tissue, the amidation of glutamic acid is worth to be noted, because it withdraws the amino acid from the GOT, GPT and ABAT action[1].

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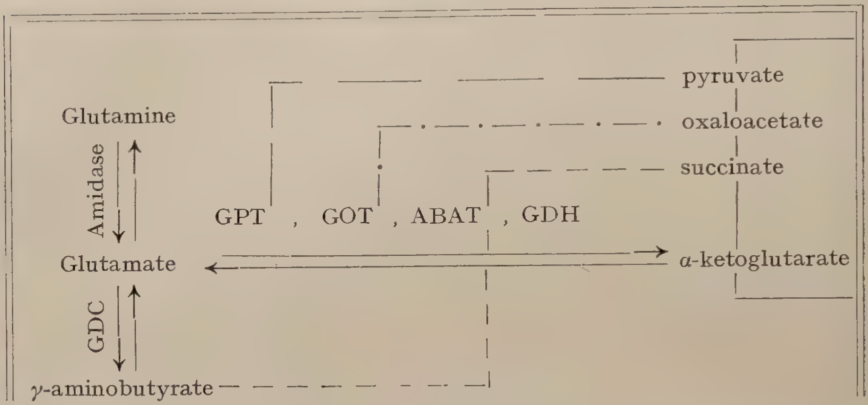
[1] Only the spleen contains almost the same amount of glutamic acid (74).

Scheme 3



The transamidination between γ-aminobutyric acid and arginine resulting in the formation of γ-guanidinobutyric acid has been omitted in scheme 3, since its metabolic significance is still unknown and the reverse reaction has not been observed (74).

The reductive amination of α-ketoglutaric acid does not operate at high speed in the brain (39), and the reverse reaction does not seem to be operating at all, since the presence of minimal concentrations of ammonia strongly opposes the oxidation of glutamate (69). Similarly, on the basis of the equilibrium constant for the transamination between glutamic and oxaloacetic acid (36), one would think that normally GOT is not a source of glutamate. The same holds for GPT.

It has been known for a long time that " *L*(+)glutamic acid is the only amino acid.... capable of mantaining the respiration of brain or retina " (38)[2]. Actually, there are two oxidation routes: the first involves direct transamination with formation of α-ketoglutaric acid, while the alternate pathway involves a preliminary decarboxylation to γ-aminobutyric acid going to succinic semialdehyde. As reported by McKhann and Tower (53), γ-aminobutyric acid is a substrate of oxidative phosphorylation in cat cortex mitochondria. It is interesting to note that with both glutamic and γ-aminobutyric acid a transamination step is required, thus making clear why pyridoxine deficiency lowers the cerebral O_2 consumption (74).

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[1] The defect of this amidation has been observed in the brain of patients with active multiple sclerosis (1).

[2] At variance with glutamic acid, aspartic acid and alanine are not substrates of oxidative phosphorylation in rat brain mitochondria (52). On the contrary, they are substrates of oxidative phosphorylation in liver and kidney mitochondria.

A lowered cerebral respiration has been reported also whenever the level of free ammonia raises in brain (see 7,52). It will be recalled that brain can use ammonia only for two reactions: glutamine synthesis and glutamic acid synthesis from α-ketoglutarate. The disappearance of this last compound " might well be injurious, since there are few mechanisms for its replacement. The Krebs cycle would be inhibited to the extent of the loss of α-ketoglutaric acid " (7). In muscle, there are quite a few reactions which could partially balance the loss of the ketoacid, but this does not hold in brain for several reasons, since in the nervous tissue the transamination between glutamic and pyruvic acids is minimal, the blood-brain barrier opposes the penetration of α-ketoglutaric acid from blood, and the fixation of CO_2 has not been detected under experimental conditions which permit carbon dioxide fixation in other organs. Therefore, the transamination between glutamic and oxaloacetic acids in the brain would seem to be the only emergency mechanism which could supply α-ketoglutarate to the Krebs cycle. The concentration of oxaloacetic acid might be, however, a seriously limiting factor. There are not data on the K_m of GOT for oxaloacetic acid. It has been reported (65), however, that the K_m for α-ketoglutaric acid is 1/5 the K_m for $L(+)$aspartate in the case of GOT from rabbit brain. If a similar ratio exists between the K_m's for oxaloacetic and glutamic acid, the rate of the transamination between glutamic and oxaloacetic could be high, even when the concentration of oxaloacetic is definitely lower than the concentration of glutamic acid.

Despite the emergency mechanisms outlined above, the brain remains exceedingly inefficient in any attempt to reduce the increase in free ammonia, and GOT must be regarded as a " buffer system capable of accomodating *only partially* a sudden influx of ammonia and of ensuring its gradual disposal during periods of restitution " (78).

As a conclusive comment on scheme 3, it is worth noting that probably glutamine is not a source of glutamic acid, although the ratio between the maximal rates of glutamine synthesis and glutamine hydrolysis *in vitro* is lower than 1 (52). A physiological role of glutamine splitting as a source of glutamic acid (35) is indeed highly questionable.

It is out of the aim of the present paper to summarize all the metabolic pathways of *L*(+)glutamic acid (see for references, 74, 78), but some other remarks cannot be avoided. In fact, the metabolic position of transaminases in the nervous system can be more fully illustrated through an analysis of the numerous and peculiar relationships of *L*(+)glutamic acid with other compounds in the brain. Thus Layne and Bessman (41) have reported that the addition of 0.01 M *L*(+)glutamate to rat brain homogenates, in the presence of succinate, ATP and DPN, increases the oxygen consumption and produces a five-fold increase of α-ketoglutaric acid. That these findings apply to an *in vivo* system appears likely, since as shown in Table III, the concentration of *L*(+)glutamic acid in the brain is 0.01 M/kg.

Recently Strecker (70) has summarized the relationships between *L*(+)glutamic acid and carbohydrate metabolism. Compounds which have known action on carbohydrate metabolism also affect *L*(+)glutamate and related compounds, as shown by the following results: 1) rat brains poisoned with fluoroacetate contain more alanine and less glutamate than controls; 2) after thiopenthal anesthesia rat brains contain more aspartate, a little more glutamine and less glutamate; 3) insulin produces an increase in aspartate and a decrease in glutamate (23, 24, 25, 26). Moreover, Strecker (70) has observed the stimulating action of *L*(+)glutamic acid on the glycolysis of brain mitochondria, which are the only mitochondria capable of glycolysis (52). This finding is in line with the results of investigations by Krebs and Weil-Malherbe (34, 77), who showed the stimulation of aerobic glycolysis and the inhibition of anaerobic glycolysis by *D*- and *L*-glutamic acid, glutamine and *D*, *L*-hydroxyglutamic acid[1].

The analysis of glutamic acid metabolism has been considered as a reliable clue to the study of transaminases in the brain. A further insight, however, can be obtained by following the metabolic fate of other compounds which can undergo transamination reactions. Thus, in the citric acid cycle (see scheme 3) there are four reactions which are important to our topic, since one or two of their substrates are involved also in transamination. These four reactions are reported in Table II. The first reaction is to some extent spontaneous, but a specific enzyme speeds it up to 400 μ-moles/g/h (58), when the pH is 5.6 and the temperature 15°C.

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[1] Stimulation of aerobic glycolysis has been observed also with K^{+}_{ions} (see 70).

TABLE II. – *Reactions of the citric acid cycle, involving compounds which participate in transaminations.*

Oxalosuccinic acid $\rightleftarrows$ α-ketoglutaric acid + CO_2
α-Ketoglutaric acid + CoA $\longrightarrow$ succinyl-CoA + CO_2
Oxaloacetic acid + acetyl-CoA $\rightleftarrows$ citric acid + CoA
Malic acid $\rightleftarrows$ oxaloacetic acid

Any attempt to correlate, from a dynamic standpoint, the decarboxylation of oxalosuccinic acid to other reactions involving α-ketoglutarate is premature, since the *iso*citric acid dehydrogenation is a seriously limiting factor.

No quantitative data are available concerning the maximal rate of the second reaction in Table II. It appears to be the first step of a chain leading again to GOT through succinate, fumarate, malate, oxaloacetate. The third reaction is exceedingly active in brain, as compared to most tissues, although values observed *in vitro* are quite close to the calculated minimum. The rate reported by Coxon and Peters (22) is equal to 30 μ-moles/g/h. MDH, which catalyzes the last reaction of Table II is a very active enzyme in brain (see Table IV), but the rate of the back reaction is much lower than the rate of the forward reaction (4) [1]. Therefore, it would seem that the dehydrogenation of malic acid is a very effective mechanism to regenerate oxaloacetic acid in the nervous tissue.

Table III summarizes data concerning the molar concentration of several compounds functionally related to transaminating enzymes. Table IV gives the maximal rate of the most important transaminases and related enzymes. The data are derived from different sources and do not refer to the same species. It would be desiderable to assemble data concerning definite brain structures and definite

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[1] Fumarase is also very active, yielding about 2000 μ-moles of malic acid/g rabbit brain, when the pH is 6.8 (49).

TABLE III. – *Concentration in rat brain of several compounds participating in transamination or functionally related reactions.*

Compounds	Molar concentration [1]	References
Alanine [2]	—	—
γ-Aminobutyric acid [3]	$1.5 — 2.7 \times 10^{-3}$	(6)
Aspartic acid	3.0×10^{-3}	(52)
α-Ketoglutaric acid	1.3×10^{-3}	(52)
Glutamic acid [3]	$10.4 — 12.5 \times 10^{-3}$	(6)
Glutamine [3]	$4.6 — 5.4 \times 10^{-3}$	(6)
Oxaloacetic acid	0.64×10^{-3}	(52)
Pyruvic acid	1.96×10^{-3}	(52)
Succinic acid	0.34×10^{-3}	(52)

[1] Molar concentrations (moles/kg wet tissue) have been calculated on the basis of figures reported by different authors.

[2] The concentration of alanine in rat brain has not been measured. In cat brain the molar concentration is 0.945×10^{-3} (72).

[3] The molar concentrations of these amino acids have been determined in brain cortex.

cellular types. Therefore, Table III and Table IV represent only a generalized picture. Even in a generalized view, however, it can be seen that GOT and MDH exhibit exceedingly high activities as compared to all the other enzymes (Table IV). Since the concentration of oxaloacetic acid is quite low, and several enzymes require the ketoacid, it might well be that the concentration of oxaloacetic acid is a rate-limiting factor in the transamination with glutamic acid. Whether the concentration of inorganic phosphate in brain is another limiting factor is still unknown (see Nisonoff and Barnes, 57).

TABLE IV. – *Activity in brain of glutamic-oxaloacetic transaminase and several related enzymes.*

Enzymes	Activity (μ-moles/g fresh tissue/h)	References
GOT	1900	(52)
GPT	60	(52)
ABAT	5	(52)
GDC	19.7	(44)
GDH	90	(52)
Glutaminase	36	(52)
Glutamine synthetase	10-20	(52)
MDH	5300	(71)
Oxalosuccinic decarboxylase	400	(58)
Condensing enzyme	30	(52)

In closing the present discussion on the metabolic significance of transaminases in the nervous tissue, it seems worthwhile to comment very briefly on the transamination energetics. Transamination reactions are related to quite small free energy changes (15) as it is the case with the dehydrogenation of glutamate. Therefore, it must be concluded that "the large negative free energy change for the overall deamination reaction actually occurs during the oxydation of the reduced pyridine nucleotides and is coupled with the formation of ATP" (15). Since it has been postulated (see 15) that only the oxidative deamination of glutamate is coupled to ATP synthesis (other amino acids transfering their amino groups to α-ketoglutarate), it would appear that the transamination to α-ketoglutarate is the main linkage between the deamination processes and the dehydrogenation of *L*-glutamate.

III. – *Biochemical pharmacology of transamination reactions.*

Some aspects of the biochemistry of pyridoxal 5-phosphate will now be considered. The discussion is aimed, however, to stress only those data which give an insight into few neuro-physiological phenomena.

It has been known for a long time that epileptic convulsions can be determined in the human as well as in other species by deficiency of pyridoxine (74). Some compounds like *iso*niazid, semicarbazide, and thiosemicarbazide (74) are able to induce analogous convulsions. It was assumed (75) that the masking of the aldehyde group in the 4-position of pyridoxal 5-phosphate resulted in the inhibition of pyridoxal-dependent enzymes, which in turn would be the biochemical basis of the neurological phenomena (33). It is obvious that a reaction with the aldehyde group of phosphopyridoxal requires that this group is not involved in the binding with its apo-enzymes and/or that the aldehyde group of bound pyridoxal 5-phosphate is reactive. General discussion of the binding between pyridoxal 5-phosphate and apo-proteins will be omitted in this report, but it is worth noting that the carbonyl group of pyridoxal 5-phosphate seems to be involved in the binding to different apo-enzymes (see for references, 11). Some results concerning *iso*niazid and transaminases, however, deserve a comment. It has been shown that the *iso*nicotinoylhydrazone of pyridoxal 5-phosphate (Py5PINH) is capable of activating the apo-protein of GOT from pig heart (13) and from human brain (10), ant it seems that a displacement reaction of the following kind takes place:

$$\text{Enzyme.Py5PINH} + \text{L}(+)\text{aspartic acid} \rightarrow$$
$$\rightarrow \text{Enzyme.Py5P.L}(+)\text{ aspartic acid} + \text{INH}$$

with appearance of the native coenzyme through the formation of the enzyme-substrate complex.

The coenzymatic role of Py5PINH drastically contrasts, at least in the case of convulsions due to *iso*niazid, the hypothesis reported above, although much caution is needed in the evaluation of results obtained with resolved GOT (see also 17). However, Py5PINH behaves like the native coenzyme also with arginine apodecarboxylase (11). The inhibition of GOT by *iso*niazid, as reported by Jenkins *et al.* (31), does not contrast with the mentioned coenzymatic role of Py5PINH. *Iso*niazid is, in fact, a competitive and extremely weak inhibitor of GOT (31). Conversely, the finding of a competitive inhibition ($K_i = 1.4 \times 10^{-2}$ M) is consistent with the displacement reaction postulated by Bonavita and Scardi (13) for Py5PINH. Beside the instantaneous and competitive inhibition, there is also a slow and non-competitive inhibition which takes place only in the presence of enormous amounts of isoniazid (0.2 M) and if pH is below 7. This concentration does not occur *in vivo*, and the inhibition of non-competitive type has no physiological significance. In conclusion, the results obtained by Bonavita and Scardi (13) with Py5PINH raise a serious objection to the hypothesis of hydrazone formation as a mechanism of transaminase inhibition. Moreover, *iso*niazid unlike other carbonyl reagents has been shown to be a very weak inhibitor also of other pyridoxal-dependent enzymes (33). Therefore, the biochemical basis of convulsions by *iso*niazid does not seem to be a simple direct inhibition of GOT, ABAT, GDC and related enzymes. A new hypothesis can be derived from the observations of McCormick and Snell (50) on pyridoxal kinase from human brain. These authors have reported a strong inhibition of this enzyme with different carbonyl reagents and their condensation products with pyridoxal, the K_i's of some derivatives in the brain kinase system being lower than the K_m of pyridoxal. Even more interesting is the fact that preliminary comparisons have shown that GDC from beef brain requires almost 1000-fold greater concentrations of carbonyl reagents to be inhibited than does the kinase. Therefore, the most obvious conclusion is that the main effect of carbonyl reagents is the inhibition of pyridoxal phosphorylation. As a consequence, less pyridoxal phosphate becomes available as coenzyme. The reaction of carbonyl reagents with bound pyridoxal phosphate would be only a minor pathway in decreasing the activity of pyridoxal dependent enzymes.

The data on *iso*niazid represent the most interesting results concerning the inhibition of transaminases, since all the compounds tested by Baxter and Roberts (5) on ABAT did not show any inhibition of the enzyme activity. It should be noted that the authors performed a random screening of very different compounds like strychnine, procaine, serotonin, reserpine, chlorpromazine, chloral hydrate, benactyzine hydrochloride, acetazolamide and iproniazid. The latter compound as well as D, L-penicillamine did not inhibit GOT from human brain (9), which in turn is strongly inhibited by D-cycloserine. This compound was tested since it is known to act as a convulsive agent and because of its ability to chelate metallic ions (9).

In connection with convulsant agents it will be recalled that injections of toxopyrimidine (4-amino-5-hydroxymethyl-2-methylpyrimidine) are capable of reducing the GDC activity in brain, while do not determine any inhibition of brain GOT and GPT (61, 62).

Investigations with compounds, previously shown to be inhibitors of transaminases, do not give any evidence of significant differences between GOT and ABAT from brain and other sources. Hydroxylamine, p-chloromercuribenzoate and N-ethylmaleimide are the most powerful inhibitors of ABAT. Phenylmercuric acetate and maleic acid are the strongest inhibitors (after D-cycloserine) of GOT, while iodoacetic acid does not depress the activity of the latter enzyme. It may be concluded that, although thiol groups are essential for the activity of GOT, they are differently accessible to iodoacetic acid and phenylmercuric acetate. The biochemical pharmacology of transaminases should take into account the data on thiol groups and especially the low reactivity of the aldehyde group in bound pyridoxal phosphate with compounds different from the natural substrates, such as azide and cyanide (5).

This review is mainly aimed at showing that many problems are still unsolved and that investigations along widely divergent lines can still contribute to explanation of biological functions in this field.

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Arch. ital. Biol., *99*: *191-211*, *1961*.

JOHN FARQUHAR FULTON

1899 - 1960

The *Archives italiennes de Biologie* wish to express deep regret concerning the recent death of Professor John Farquhar Fulton. His contributions to science have been outlined by one of his former pupils, Dr. A. Earl Walker, in the *Journal of Neurophysiology*, which professsor Fulton created and edited up to the end of his life.

For many years people from all parts of the world came to his laboratory, for inspiration and guidance in the filed of Neurophysiology. Being a stimulating leader with a warm, generous personality, Prof. Fulton engendered affection and loyalty in all those who worked with him. His friends and pupils throughout the years will not forget him.

We are pleased to report that former fellows and collaborators of Professor Fulton intend to honor his memory by creating the Fulton Society, in which anyone who had the privilege of working with him and enjoyed his kind friendship will meet to maintain his name and ideals alive in the scientific circles to which he devoted his life. Those who are interested and have not yet received information, should write to Prof. Dr. Victor Soriano, Calle Buenos Aires 363, Montevideo, Uraguay.

ANALYSES

Brindley, G. S. *Physiology of the retina and visual pathway.* London, Ed. Arnold, 298 pp., 35 s. (Monographs of the Physiological Society series).

The enormous and semicoherent mass of observation and opinion which bears upon the physiology of vision has been distilled to an essence of only 250 pages, a strong draught with a distinctly astringent flavour. How has such concentration been achieved? Dr. Brindley has made a considerable effort to discover the original author of all the observations quoted, and has left unmentioned subsequent authors unless they did substantially more than repeat work already well done. Nor has he space for the flotsam of findings from which nothing in particular can be deduced. Thus perhaps ten papers have been passed unmentioned for each one of the 1,000 listed in the References.

Significant observations are compactly presented and their inferences closely argued, but never in the spirit of controversy. The topics of vision have always teemed with dispute, but the reader will find in this author no eloquent advocate but rather a judge whose object is to state briefly but precisely how far the case is proven. The book consists of seven chapters.

1. *The photochemistry of the retina.* — The inherent difficulties of this subject are increased because authorities are not agreed upon some important facts or upon nomenclature. Brindley sets out plainly the evidence and conclusions where there is no dispute and summarized the conflicting evidence where controversial.

2. *The electrical activity of the retina* is divided into E.R.G., other slow electrical activity and spikes in ganglion cells and elsewhere. The long puzzle as to the nature of the E.R.G. is not yet solved, but analysis with the micropipette has thrown much light upon it, Brindley's own work being outstanding. His treatment of the whole subject in this chapter is probably the clearest analysis which has ever been given. The rest of the chapter summarizes what is known of ganglion discharges and other nerve activity.

3. *The central pathways of vision.* — Our fundamental ignorance about the physiology of brain organization makes this attempt to assess the function of the various optic pathways the least satisfactory analysis in the book. As Brindley points out, the superior colliculi have no known function to warrant a sensory input of 250,000 optic nerve fibres. It is not known what advantage is scored by nerves from retina to cortex having a relay at the lateral geniculate. Nor what sensory function could be served by centrifugal fibres in the optic nerve which would justify the precious space they occupy. A great deal of information has been obtained in relation to vision by recording from the brain or by partly destroying it, but they lead to no clear picture of what the brain does with the visual message.

4. *Introduction to sensory experiments.* — Though every scientific observation and measurement ultimately makes use of sensory perception, in nearly every case this amounts simply to the detection of a coincidence upon some scale in a frame of reference, and almost any sense organ could

in principle be used as detector. This, however, is not the case in the study of sense organs themselves through their perceptual effects, for here we are concerned not with coincidences outside us, but with the actual relation between stimulus and perception. In 6 pages Brindley examines the logic of this situation and divides sensory observations into two classes. Class *A* is based upon the assumption that optic nerve signals which are physically identical will result in identical visual sensations. All sensory observations which are not concerned simply with judging the identity of a pair of sensory experiences are called " Class *B* " observations, and though it is usually extremely hard to argue soundly from them, some ways of doing so are discussed.

5. *Some sensory experiments involving neither matching nor threshold.* — This deals with such well established class *B* phenomena as the entoptic images of the shadows of retinal vessels, Maxwell's spot, simultaneous and successive contrast, electric phosphenes, etc.

6. *Spatial, temporal and adaptational aspects of the visual threshold.* — In a fixed state of adaptation the interplay of wave length, area, flash duration and background are discussed in relation to threshold, with flicker and acuity measurements as particular cases. The way in which these alter in light and dark adaptation is then treated in relation to the bleaching and regeneration of visual pigments. The chapter ends with a lucid summary of the present position as to the quantum requirement of the receptors and of the whole eye at threshold.

7. *Colour vision.* — First there is a short and original historical account of the discovery of trichromacy. Then a brief but very general treatment is given of the classes of hypotheses which are consistent with trichromacy, and their implications. The stability of metameric matches under adaptation to coloured lights, and their breakdown under intense adaptation have important consequences which are closely argued. The spectral sensitivities of the 3 colour mechanisms is not yet settled, but evidence is taken from colour blindness, from Stiles' two-colour thresholds, from Brindley's artificial monochromasies produced by intense bleaching, and from objective measurements of the foveal pigments by retinal densitometry.

In this book are collected the key observations upon which the science of vision has been built, but the book is far more than a collection. For Brindley not only gives us the evidence; he teaches us to think.

W. Rushton

Bindra, D. *Motivation: a systematic reinterpretation.* New York, The Ronald Press Company, 1959, VII-361 pp., $ 4.50.

The problem of motivation lies at the core of modern psychological thinking. Motivational concepts pervade virtually all of the major areas of psychology: learning, perception, emotion, thinking, the study of instinctive behavior and so on. As a result of the remarkable growth during the past decade of experimental data and theoretical ideas concerning motivation, there has been a need for a thoughtful analysis of the problem in order to bring the facts together in a coherent, meaningful way and to provide the guidelines for fruitful future investigation. This need has been met in a highly satisfactory way by Bindra's *Motivation: a systematic reinterpretation.*

Investigators who are working in the field of " motivation " or related fields will be grateful for Bindra's book. So many publications appear each year that are germane to the problem that it has become exceedingly difficult to follow the thread of thinking and research by the many students in the field. But Bindra's book is more than a thoroughly competent review of the literature: it provides a systematic reinterpretation of much of the material, including a great deal that has never before been discussed under the rubric of "motivation." The formulation is broad in scope, and lends itself as a guide to the problem of motivation for both psychologists and physiologists. The treatment of the problem is psychological in nature, but a great deal of relevant physiological material is included, and physiologists interested in psychological problems will find the book especially valuable for its remarkably clear exposition of a topic that is muddled with almost-incomprehensible theoretical controversy.

The book is organized into four major sections. In the first (Chapters 1 and 2), Bindra reviews the historical development of the field and then poses the questions that characterize the problem of motivation. Although motivation is predominately a psychological conception, its theoretical development has been influenced to an astonishing degree by physiological findings. It was the early work on nerve-muscle preparations as well as the experimental analysis of conditioned reflexes by Pavlovian methods that provided psychology with the notion that behavior consists of a series of responses to stimulation. The central nervous system was considered to be normally inert unless triggered into activity by interoceptive or exteroceptive stimulation. It was in this atmosphere that instinct doctrines and drive theories of motivation flourished. Stimulation, such as hunger or pain, pushed the organism into activity, and the organism pursued a particular goal that satisfied the appetitive part of the instinct or that reduced the drive. Inherent in these notions is the idea that the organism is born with the ability to recognize the appropriate goals and with the motor responses ready-made for goal-pursual.

Within recent years, however, largely due to Hebb's *Organization of Behavior*, a fresh approach has begun toward the problem of motivation. Specific drives, Hebb pointed out, need not be postulated to account for the activity of organisms; modern neurophysiology (particularly electroencephalography) shows that the central nervous system is continuously active, so that the question "Why is the organism active at all?" can be answered in terms of the biological properties of living tissues. The chief problem for the psychologist is not the arousal of activity but its patterning and direction.

It is with the latter problem, then, that Bindra concerns himself, and he proceeds to outline two general questions that constitute the essence of the field of motivation: "The first general question is concerned with the *origin* of directed activities. How are responses patterned into complex 'purposive' activities? What factors determine their development in the animal's repertoire? The second question concerns the *occurrence* of directed activities. How is it that certain types of directed activities occur at a particular time and others do not? What specific factors affect the frequency, rate, and timing or periodicity of the various classes of directed activities? These two questions incorporate all the problems that are usually discussed under the heading of motivation" (p. 18). Bindra subsequently goes on to describe the classes of bahavior that lend themselves to analysis in terms of these two broad questions: general activity, exploration, problem-seeking and play behavior, withdrawal and aggressive activities (which are normally considered as "emotion," but which, as Bindra rightly argues, cannot be logically separated from "motivational" phenomena), eating, drinking and sexual activities, and maternal behavior.

In the second section of the book, Chapters 3 to 5, Bindra analyzes the above classes of behavior in terms of the first question, regarding the origin and development of goal-directed activities. Goal-direction, Bindra argues, is acquired, in mammalian species at least, so that "some degree of repeated or continuous exposure to a situation is a necessary condition for the development of activities that are goal-directed with respect to some feature of the situation" (p. 64). In the course of repeated exposures, behavior then "becomes goal-directed by virtue of the fact that certain movements or component responses.... accompanied by a given goal are selectively strengthened and reinforced" so that they increase in frequency in the presence of the goal, giving rise to an integrated, goal-oriented response (p. 67).

Bindra's discussion of the development of motivational activities makes fascinating reading, particularly the section on hoarding behavior, to which Bindra has himself made classic contributions. By drawing selectively from a large literature, Bindra skillfully illustrated his argument that goal-directed activities do not suddenly appear in the behavior repertoire of organisms but rather *emerge* from a diffuse, undifferentiated kind of behavior, or from previously acquired responses which may then merge to give rise to goal-directed behavior. Bindra carefully notes, however, that constitutional (inherited) differences must undoubtedly play a role in the degree of exposure necessary for a given goal-oriented response to appear and in the characteristic patterning of the responses themselves.

The Chapter dealing with an analysis of reinforcers is written with great clarity and stands in striking contrast with most expositions of the nature of reinforcement. This field is fraught with controversy, and Bindra has done a splendid job in pointing out the essential differences among the various schools of thought, the nature of the controversies, and concludes the section by drawing from all of the material to delineate the nature of a reinforcement mechanism which would be capable of accounting for most of the available data.

The third section of the book, Chapters 6 to 9, deals with the conditions involved in the occurrence and performance of goal-directed activities. These include the variables involved in the development of the strength of a response, the role of sensory cues in the guidance of motivated behavior, the concept of arousal in relation to the field of motivation, and the role of humoral and hormonal agents in the maintenance of such goal-directed activities as sexual and maternal behavior. The chapter on arousal and behavior is of especial interest to physiologists. Bindra notes that psychological investigators have used a number of criteria for "arousal" — indices of autonomic-visceral functions, of somatic-skeletal functions, and of activity in the central nervous system — and these measures are interrelated in a complex (and not always predictable) manner. There is a multiplicity of variables that contributes to the dimension of behavior called "level of arousal," and Bindra draws from data in human experimental psychology, physiological psychology and neurophysiology in developing a working concept of "level of arousal" that is sufficiently broad to account for the complicated relationships between the various measures of "arousal" and behavior patterns of diverse complexity.

In the final section of the book, Chapter 10, Bindra summarizes his approach to the problem, and deals with the problem of motivation at the human level. He draws cautiously from conclusions based on animal experimentation, and outlines an approach to human motivation that is consistent with investigations carried out in related biological sciences. As Bindra points out, the salient terms in the field of motivation are still vague, and many of the problems are just beginning to be understood. There can be no question that the field of motivation is greatly clarified as a result of Bindra's work. He shows where the problems lie and how they can be handled experimentally. His book is bound to influence the course of experimental work on motivation during the coming years.

R. MELZACK

HARLOW, H. F. and WOOLSEY, C. N. (Eds.) *Biological and biochemical bases of behavior.* Madison, University of Wisconsin Press, 1958, xx-476 pp., $ 8.00.

The aims of a symposium, to be useful both to the participants and to the readers of the printed record, are perhaps contradictory; certainly, in attempting to make a symposium valuable for both groups the organizers and editors shoulder a heavy burden. Since the present review deals with the book and not with the symposium, the point of view is that of a reader, not a participant.

We learn from the introduction that the comments prepared by the discussants and the transcripts of the general discussion were omitted in order to keep the cost of the final volume within reason. Since the symposium had an interdisciplinary approach, one may suppose that some degree of correlation and coordination of the results obtained with different techniques was achieved in the discussion. The quality of the contributions was high, certainly such as to engender creative discussion, and it is regrettable that the reader had to be deprived of tnis important part of the symposium.

Also the absence of summarizing statements of the type contributed so effectively by Gerard to the Ford Hospital symposium on the reticular formation is deeply felt. A sketch of the broad outlines of the conference and an assessment of the results of cross-disciplinary conversation would have greatly helped the reader.

Limitations of space and of the reviewer's aptitude make it impossible to provide comment on all papers, but remarks on some salient points are offered here. Harlow points out that the behavioral methods in interdisciplinary research are frequently out-of-date; the experimental psychologist is often in a position to contribute tests of higher reliability. Interdisciplinary research leads not only to new correlations, but to new data for the individual disciplines concerned. Meyer recommends a break from the traditional methodology of studies of brain lesions, offering an intriguing justification: if we take into account the costs of our research (time, money, effort), then the carrying out of elaborate controls may not always be justified. Meyer demonstrates that overemphasis on histological control, for example, may keep an experimenter from study of less conventional variables such as ones involving the experience and incidental learning of the subjects. Pribram divides the cerebral cortex into extrinsic and intrinsic spheres according to whether the input to an area comes primarily from extracerebral or intracerebral sources. His contribution is chiefly the classification and clarification of vocabulary, and may be considered more a prelude to theory than theory itself; it would be of some interest to know the conferees reacted. Rose and Woolsey present a detailed series of experiments on the thalamic relays of the cat's auditory system, incorporating anatomical, electrophysiological, and behavioral investigations. This thorough study is an outstanding example of a crossing of methological boundaries but does not yet provide the convergence of conclusions that one might hope for. For instance, no conclusions about the relations of the Rose and Woolsey research to the work by Neff and Meyer appear in the report, despite the fact that all four were present at the meetings.

The contributions of Jasper, Brady, Olds, and Riesen all deal with research which was already partly known from reports in various scientific journals. Expressions of orientation have not been so readily available however, and this volume makes a significant contribution in revealing some of the principles guiding these energetic investigators.

The conference closed with a return to the problem of interdisciplinary investigation and theorizing, presented in persuasive fashion by Hebb. Having earlier admonished the psychologist to pay heed to contemporary neurophysiology rather than an oversimplified and outdated picture of neural function, he turns here to the necessity for the neurobiologist to penetrate in some depth the substance of modern psychology, both its theory and its data. The behavioral scientist in turn is exhorted to abandon his efforts to construct oversimplified schemes of behavior, developing instead adequate formulations which can provide areas for fruitful common discussion with physiologists, biochemists, and neuroanatomists.

C. J. Smith

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Direttore responsabile: Prof. Giuseppe Moruzzi
Editore: F. Vallerini - Pisa
STABILIMENTI TIPOGRAFICI «E. ARIANI» E «L'ARTE DELLA STAMPA» - FIRENZE
Autorizzazione del Tribunale di Pisa N. 20/1956 del 1° Dicembre 1956